Exploring Factors Driving Organic Carbon Burial and Storage in Small Constructed Ponds: An Experimental Approach

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“The terrestrial biosphere is assumed to take up most of the carbon on land. However, it is becoming clear that inland waters process large amounts of organic carbon and must be considered in strategies to mitigate climate change”

Battin et al., 2009

Abstract

The significance of ponds in the terrestrial carbon cycle has received increasing attention in recent years. Evidence suggests that ponds exhibit rates of biogeochemical cycling orders of magnitude greater than larger water bodies and, cumulatively, the storage of organic carbon (OC) in small ponds may equal or possibly surpass that of large water bodies.

This project furthers our understanding of OC storage within ponds, combining survey and experimental approaches to accurately quantify sediment carbon stocks and accumulation rates. It incorporates four distinct, yet complimentary components aiming to: i) Evaluate the accuracy of estimating OC storage in entire ponds from sediment cores; ii) Monitor physicochemical dynamics and quantify OC storage across newly constructed ponds; iii) Quantify OC storage across a suite of mature ponds and assess the impact of vegetation community development; iv) Identify variations in microbial communities between different ponds in relation to sediment physicochemistry and vegetation coverage, exploring the implications for OC storage and burial.

Carbon stocks were surveyed in 12 mature ponds across an experimental field site at Druridge Bay, Northumberland. Comprehensive historical ecological data was used to separate these ponds into three distinct groups based on hydrology and vegetation. One pond was selected from each of the resulting three groups and exhumed in its entirety to accurately quantify OC storage. Three sediment cores were taken beforehand in a novel attempt to evaluate the percentage difference between sediment core estimates and whole pond sediment OC storage. Whole pond exhumation suggests that the three ponds stored between 1565 – 2288 g OC m^2, whilst results from the cores alone gave estimates within a 10-15% range. A further three ponds from each group were selected to quantify burial rates using sediment cores. Results suggest the ponds have stored between 1413 – 4459 g OC m^2 over 20 yrs, equating to around 67 – 212 g OC m^2 yr^{-1}. OC storage was greater in ponds that had undergone the fastest establishment of vascular plant communities.

Three new ponds were constructed at the Druridge site. Physicochemical parameters were monitored at approximately fortnightly intervals across a period of three years. All three ponds were hyper-eutrophic, dominated by algae. However, OC storage was negligible, further suggesting that OC burial is only significant upon the establishment of vascular plant communities.

Sediment samples were subject to 16s rRNA analysis to identify microbial communities involved in carbon cycling. Variations in microbial community composition between ponds were observed and showed complex relationships with sediment physicochemistry and vegetation coverage. Microbial diversity was significantly higher in ponds storing more OC. Ponds dominated by *Juncus* vegetation, had lower diversity and a greater abundance of facultative anaerobic bacteria, and stored less OC.

The intense rates of OC burial observed in this study demonstrate the functional capacity of constructed ponds to operate as significant sinks of OC. High rates of OC accumulation compared to the surrounding terrestrial landscape highlights the potential for their construction across landscapes to act as versatile, yet effective carbon mitigation features.

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List of Abbreviations and Terms

% RSD - % Relative Standard Deviation
%Moisture – Moisture Content
16s rRNA – 16s Ribosomal RNA
Al - Aluminium
ANOVA – Analysis of Variance
ANOSIM – Analysis of Similarity
CAP – Common Agricultural Policy
CCA – Canonical Correspondence Analysis
CH_4 – Methane
CO_2 – Carbon Dioxide
C:N Carbon to Nitrogen Ratio
C Stock – The mass of carbon (g C m^2) stored in pond sediment
DBD – Dry Bulk Density
DCA – Detrended Correspondence Analysis
EA – Elemental Analysis
EFA – Ecological Focus Area
Fe - Iron
GHG – Greenhouse Gas
GPP – Gross Primary Production
K - Potassium
IOC – Inorganic carbon
LoD – Limit of Detection
LOI – Loss on Ignition
LoQ – Limit of Quantification
Mg - Magnesium
N – Nitrogen
NEP – Net Ecosystem Production
NGO – Non Government Organisation
NO_3^- - Nitrate
OC% - Organic Carbon Concentration in Sediments
OC – Organic Carbon
OM – Organic Matter
OTU – Operational Taxanomic Unit
P – Phosphorus
PCA – Principal Component Analysis
PO_4^{3-} - Phosphate
S – Sulphur
Si - Silicon
SD – Standard Deviation
SE – Standard Error
TWINSPAN – Two-way Indicator Species Analysis
SIMPER – Similarity Percentages
WFD – Water Framework Directive

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Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. I also confirm that this work fully acknowledges opinions, ideas and contributions from the work of others.

Any ethical clearance for the research presented in this thesis has been approved. Approval has been sought and granted by the Faculty Ethics Committee on 21/04/16

I declare that the word count of this thesis is: 54,264

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Signature:…….....

Date:…….....

Publications and Communications

Publications

Gilbert, P., M. Jeffries, D. Cooke, M. Deary, S. Taylor. 2016. Quantifying rapid spatial and temporal variations of CO_2 fluxes from small, lowland freshwater ponds.. *Hydrobiologia*.

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To be Submitted

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Reports

Natural England and the Northumberland, Yorkshire, and Norfolk Wildlife Trusts: A summary of works conducted on ponds across England and their sediment carbon stocks. *Report to be compiled for communication of works conducted with associated partners and to inform pond management practice.*

Northumberland Wildlife Trust (07.05.2014) The value of small aquatic systems beyond conservation and biodiversity. *Contributions to a report & presentation to estates manager of the trust on our research conducted on their sites over the previous 3 years.*

Conferences

Symposium of European Freshwater Sciences (SEFS) – Geneva – July 2015 – Oral Presentation: *Elucidating ecological controls underpinning carbon storage in small water bodies: An experimental approach*

Society of Wetland Scientists/ European Pond Conservation Network (EUPCN) – Huesca, Spain – September 2014: *Ponds, Probes and Productivity: An experimental approach*

British Organic Geochemistry Society (BOGS) – Plymouth – July 2013 – Poster Presentation: *How effective are small artificially constructed ponds at sequestrating and storing atmospheric carbon*

Northumbria University Research Conference – Newcastle – April 2013 – Poster Presentation: *Organic carbon burial in small constructed ponds*

Chapter I – Contextual Review into the Role of Small Ponds in the Global Carbon Cycle

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Project Overview

1.0 Introduction

The creation of the United Nations Framework Convention on Climate Change (UNFCCC) in June 2006, placed increasing global importance on climate change. The primary objective of the UNFCCC was the "stabilization of greenhouse gas concentrations in the atmosphere at a level that would prevent dangerous anthropogenic interference with the climate system" (Houghton, 2007). The framework outlined the importance of developing our understanding of key processes, such as how much anthropogenic carbon released remains in the atmosphere and how much of this emitted carbon is taken up by the world's oceans and terrestrial ecosystems (Houghton, 2007)

More recently, the 2015 Paris Climate Change Conference, also known as COP21, resulted in 195 countries signing a legally binding agreement to tackle global warming. It has been described as a "momentous, world-changing" agreement with the primary goal of member parties being to hold temperatures to;

> *"well below 2°C above pre industrial levels and pursue efforts to limit temperature increase to 1.5°C above pre industrial levels".*

The agreement outlines a number of articles in order to achieve this, one of the most prominent being;

> *"achieve a balance between anthropogenic emissions by sources and removals by sinks of greenhouse gases in the second half of this century on the basis of equity and in the context of sustainable development..." COP 21 UNFCCC 2015 Adoption of the Paris agreement"*

Summarising the agreement, one of the key pathways to achieve this goal is by removing carbon dioxide (CO_2) from the atmosphere

> *"Society will need to continue further, to negative emissions. That is removing carbon dioxide from the atmosphere and storing it somewhere else. There are various options here, from planting trees and keeping restored forest in perpetuity, enhancing uptake in soils..."*

This places increasing importance on developing our understanding of ecosystems and the functional role they play in global carbon cycling, as either sinks or sources.

Small aquatic habitats, such as ponds and small wetlands, have, until the last 20 years, been largely overlooked in scientific research. Despite observations on ponds dating back to the 4th century BC in Aristotle's "The History of Animals" where he described

the seemingly spontaneous generation of life from rain, mud or sand (Boix et al., 2012). More recent appreciation from author Gene Logsdon in his book “The Pond Lovers” relates the life histories of people who outline emotive feelings towards water, specifically “the attraction to water is in our genes” (Logsdon, 2003).

Previous misconceptions that large lakes and rivers are more important in terms of their biodiversity, global coverage and biogeochemical processing, have developed a strong bias to the study of larger water bodies. The perception and theory that ponds and small wetlands potentially play significant roles in global processes was inconceivable and untested. An analysis of the number of pond studies in comparison to studies on larger water bodies found that they constituted only about 25% of publications in a given year (Downing, 2010).

Recent decades have seen a gradual increase in research focusing on small water bodies, particularly their ecological importance and functioning. As a result of this, their regional importance and national value, for the conservation and improvement of biodiversity, is now widely recognised by scientists and landscape managers alike e.g. (Céréghino et al., 2013; Boix et al., 2012; Ewald et al., 2012; Oertli et al., 2009; Jeffries, 2008; Biggs et al., 2005; Williams et al., 2003). More recent research has emerged, demonstrating the potential of ponds and small water bodies to play a substantial role in global carbon cycling (Holgerson & Raymond, 2016; Gilbert et al., 2014; Raymond, 2013; Downing, 2010; Downing et al., 2008). In the same way that their importance for biodiversity was not appreciated, it is possible that despite their small size, ponds may actually contribute substantially to biogeochemical cycling.

The extent, to which ecosystems contribute to global biogeochemical cycling, is based on their cumulative surface area and the intensity of processing rates. Both factors were assumed negligible for ponds and small wetlands; however, emerging research contradicts this presumption. Improved and upscaled estimates of their global distribution, alongside recent reported carbon burial and storage rates, supports the hypothesis that they may in fact process carbon at rates equal to that of the earth’s largest lakes (Verpoorter et al., 2014; Seekall et al., 2013; Downing, 2010; Battin et al., 2009; Tranvik et al., 2009; Cole et al., 2007). The remainder of this chapter is primarily focused upon these key components, providing an overview of current research on the role of ponds in global carbon cycling.

2.0 Ponds

Ponds are familiar habitats to many people, as part of rural and urban landscapes alike. Individual perceptions on ponds reflect people's personal experience with these habitats and will no doubt vary considerably depending upon the ponds appearance and function (e.g. size, vegetation coverage, commercial fishing, ornamental fish keeping or water features). This is unsurprising as ponds are ubiquitous features throughout landscapes at regional, national and international scales. They are found throughout the majority of the earth's terrestrial biomes, from the high Arctic (Abnizova et al., 2012), to Antarctica (Allende & Mataloni, 2013) and occur throughout natural, agricultural and urban landscapes (Chou et al., 2014; Hassall, 2014; Heckenberger et al., 2007)

Many of these features are formed via natural geomorphological processes, such as glacial Pingo and Kettle Hole ponds, though a large proportion have been constructed for specific anthropogenic activities, such as livestock watering, fishing or wildlife hunting. These features are also created inadvertently from anthropogenic activity, for example subsidence ponds in North East England and small ponds created from the removal of anti-tank barriers at Aberlady Bay, Scotland (Jeffries, 1998).

For many years the defining boundaries of what constitutes ponds, small lakes, small wetlands and pools have been somewhat blurred. Early definitions were based on four broad categories: i) it is difficult to describe a pond; ii) ponds are small and shallow; iii) ponds are shallow enough for rooted plants to grow throughout; iv) a miscellany of other physical characteristics. Some studies argued, "There is no point at which a definitive line can be drawn between a pond and a lake or even between a puddle and a pond" (Fitter and Manuel, 1986). Other early definitions were extremely broad such as "A smaller version of lakes" or "a pond is anything less than 50m or so across..." (Beebee, 1991; Moss, 1988). The first attempt at producing a simple standardised sized based definition was adopted in the early 1990's. Ponds were defined as:

"Water bodies between 1m^2 and 2 ha (20,000m^2) in area which may be permanent or seasonal including both manmade and natural water bodies"

This has since become widely accepted and utilised in recent literature, most notably by the RAMSAR convention on wetlands (Biggs et al., 2005; Angélibert et al., 2004). Although simplistic, the definition is broadly inclusive covering the wide range of small

aquatic systems that may have previously been discounted based on size, depth, permanence and origin.

Discounting ponds based on such variables seems foolish especially given our current understanding. Many are shallow systems, however those constructed for agricultural purposes (Downing et al., 2008), and even some natural features, have depths beyond the criteria of previous definitions. Temporary ponds form a large proportion of overall pond numbers, yet classifying these features as puddles has led to them being overlooked as valuable habitats. Their inclusion under this definition alongside growing research has highlighted their value for a whole host of invertebrate, plant and amphibian species, supporting communities distinct from permanent ponds (Williams et al., 2010; Nicolet et al., 2007, Nicolet et al., 2004; Williams et al., 2001). Furthermore, the inclusion of anthropogenic water bodies means that ponds created for specific human activities or wildlife conservation, are included in important regional and national surveys, especially considering many are not fundamentally ecologically different from "natural ones" (Céréghino et al., 2013; de Marco et al., 2013).

Ponds are still included under other terms in scientific literature such as "shallow lakes" (Moss et al., 2009; Scheffer et al., 1993) or "Wetlands" (Van der Valk, 2006; Gopal et al., 2000), particularly in the USA where the term wetlands is often used to describe ponds and other small aquatic systems. General definitions may explain some of the bias observed in scientific literature and current policy, such as the study of lakes and wetlands over ponds, and their inclusion within the EU Water Framework Directive. (Boix et al., 2012; Miracle et al., 2010).

It was not until the 1990's that the value and importance of ponds as wildlife habitats was truly appreciated by freshwater scientists. The formation of NGO's such as the Freshwater Habitats Trust (formerly Pond Action), that focus on conserving the ecology of these habitats, facilitated further research, whilst also providing a platform for scientists, land managers and policy makers to communicate scientific and practical understanding. As such, their value in the conservation of biodiversity at a regional scale, supporting substantial numbers of nationally scarce and red data book species in comparison to rivers and lakes (see tables I.1 and I.2), is now widely recognised. The heterogeneity of pond types and superficially similar ponds over local and regional networks facilitates high gamma diversity (*y*), with ponds supporting a greater number of species than lakes, streams and rivers (Céréghino et al., 2013; Oertli et al., 2009).

Table I.1: Pond and river invertebrate species richness and rarity comparison, taken from Biggs (2005).

	Ponds (200)	Rivers (614 sites)
Number of species	431	377
Nationally Scarce species (occurring in 15-100km squares)	78	41
Red Data Book species	26	13

Table I.2: Pond and lake aquatic plant species richness and rarity comparison, taken from Biggs (2005).

	Ponds (200)	Lakes (1100 sites)
Number of species	72	89
Nationally Scarce species (occurring in 15-100km squares)	7	8
Red Data Book species	5	5

2.1 Temporary Ponds

As outlined in the definition of a pond (Biggs et al., 2005), these systems can be seasonal, or temporary. Temporary ponds are described globally under a suite of different names e.g. vernal, ephemeral and playas (Tangen et al., 2015; Goldyn et al., 2015; Kneital, 2014; Keeley & Zedler, 1998). Many ponds across the smallest size ranges are subject to marked seasonal changes in surface area, or even complete drought events, whereby ponds dry up completely. This can happen every few years, annually or even several times within a single season depending on local climate and its relation to pond morphology and local hydrology. For many temporary ponds, recharge is often rainfall dependent. Wetting and drying is therefore heavily reliant on the balance between local rainfall and evaporation rates over monthly periods (Reverey et al., 2016; Jeffries, 2015; Sahuquillo et al., 2012; Dimitrou et al., 2009). Drying events often leave the pond substrate exposed resulting in desiccation of sediment layers, creating complexities in the biogeochemical cycling of carbon and nutrients throughout transitional periods (Revery et al., 2016; Gilbert et al., 2016).

Temporary ponds potentially make up a substantial proportion of the global pondscape. However, their presence is often overlooked in both natural and agricultural landscapes due to limitations in remote sensing, satellite imagery and the possibility of being dry when surveyed (Williams et al., 2010). In the UK alone at least a quarter of lowland ponds are shallow water bodies subject to reoccurring dry phases (Williams et al.,

2010; 2001; 1998). In warmer climates this is likely much larger, however, accurately quantifying the number and surface area of these features is limited by their seasonal presence.

The temporary nature of these features means they have long been overlooked as valuable habitats for aquatic and terrestrial species. Many species of plants, macroinvertebrates and amphibians are well suited to semi aquatic and seasonal drought conditions, resulting in temporary ponds supporting ecological communities distinct to features that are more permanent. Many provide habitat for uncommon and rare species (Nicolet, 2004; Collinson et al., 1995; Prestion, 1989), and are important for terrestrial and semi terrestrial invertebrate groups (Drake, 2001; Lott, 2001). It has also been reported in a survey of 71 temporary ponds, 75% supported at least one uncommon, nationally scarce or red data book species. Almost three quarters supported one nationally scarce macroinvertebrate and 8% supported one nationally scarce plant species (Nicolet et al., 2004). These studies paved the way for ponds, such as Mediterranean Temporary Ponds, to be classified as priority habitats and integrated into policy such as the EU Habitats Directive (Céréghino et al., 2013; 2008).

Inconsistent annual rainfall patterns and projected future climate variability, resulting from climate change, creates uncertainty on the future functioning of temporary systems. Of particular note is the conservation of temporary freshwater systems and loss of valuable pond networks, such as Mediterranean Temporary Ponds (Zacharias et al., 2007). It also complicates attempts to model and quantify biogeochemical dynamics, making it difficult to comprehensively understand and predict ecosystem functioning within these systems.

3.0 Environmental Policy

Despite increasing recognition of the value of ponds, they receive limited protection from current legislation and policy. Ponds with historical uses are protected under Natural Heritage schemes, listed as Scheduled Monuments (e.g. moats, mill ponds, hammer ponds (Keeble et al., 2009), yet ponds in the wider landscape receive little protection, due to their small size and prioritisation of larger lakes and rivers. Even the Water Framework Directive (WFD), despite its intention to achieve "good status" for all water bodies, fails to address the status of small ponds, focusing largely on water bodies > 50,000m^2 (Céréghino et al., 2013, Boix et al., 2012, Miracle et al., 2010).

Sutherland (2006) outlined 100 ecological questions of high policy relevance in the UK, a number of which mention ponds directly, or could include ponds in the context of the question. Some examples are as follows;

1) *What are the benefits of protected habitats in terms of water resources, carbon sequestration and other, goods and services relative to non protected land?*

2) *How do current agricultural practices affect the conservation value and extent of non-agricultural habitats such as woodland edges, hedgerows and ponds, and how can detrimental impacts be mitigated?*

3) *What would be the ecological implications of large scale river and floodplain restoration schemes in the UK, and would they be more cost effective than traditional hard flood defences?*

4) *How can flood control be assisted by appropriate habitat management and restoration, and what are the impacts on biodiversity?*

5) *How can soil carbon be retained and further carbon sequestered in the soil?*

Ponds have the potential to meet a variety of landscape objectives particularly in relation to biodiversity, flood mitigation, sediment retention and carbon sequestration. Answering these questions with sound scientific evidence is necessary, if ponds are to be successfully integrated into landscape policy and legislation, to both protect and promote the conservation of these habitats. Following sections will discuss the ecosystem services provided by ponds in more detail.

The formation of NGO's such as the Freshwater Habitats Trust (formerly Pond Action and Pond Conservation), that focus on the ecology of these habitats, has facilitated further collaborative research, whilst providing a platform for scientists, land managers and policy makers to communicate scientific and practical understanding. Schemes such as the Important Areas for Ponds (IAP) formulated by Pond Conservation, and the

EPCN (European Pond Conservation Network), have been established in order to conserve or create these features as part of catchment management and restoration options. Further work from the Freshwater Habitats Trust has campaigned to see these features included in key policy reports, such as the Biodiversity Action Plan, the Natural Environment White paper and the UK National Ecosystem Assessment.

3.1 The Natural Environment White Paper - The Natural Choice: Securing the Value of Nature 2011

The aim of this document is to improve the quality of the natural environment across the UK, moving to a net gain in the value of nature, arresting the decline of species and habitats under degrading landscapes. The documentation seeks to protect priority habitats and safeguard vulnerable resources for future generations, supporting the natural functioning of ecosystems in urban, rural and marine areas. The overall goal is to synergise actions at local and national levels, to create ecological networks resilient to future pressures.

Ponds are included in the paper, outlining their value and effectiveness at carrying out a range of ecosystem functions and services:

> *".....small water bodies such as ponds and ditches play a critical role in supporting ecosystem services (intercepting, storing and routing water and nutrients, transporting and transforming carbon, and supporting biodiversity)"*

Whilst also promoting their creation:

> *"Ponds alone support 70% of freshwater biodiversity and more endangered species than lakes, rivers, streams or ditches. Land managers and communities can add diversity to ecological networks in an inexpensive and highly effective way, by creating small clean water bodies across the countryside"*

The report also outlines the Flood and Water Management Act 2010, which includes the provision to increase the construction of water bodies in new developments through Sustainable Urban Drainage systems (SuDs). In addition, the Act also encourages communities to retrofit sustainable drainage in gardens and neighbourhoods such as public wetlands, rain gardens and community ponds, providing a framework for the management and maintenance of community schemes.

3.2 Biodiversity Action Plan

The UK Biodiversity Action Plan (BAP) was published in 1994 and was the government's response to the Convention on Biological Diversity (CBD) signed in Rio de Janeiro 1992. The BAP identifies priority species and habitats that are understood to be the most threatened and require conservation action.

In 2007, ponds were categorised as a new priority habitat under the BAP species and habitats review, on the basis that criteria for adoption by the BAP are met. Criterion met by ponds include; Criterion 1 - International Obligations; Criterion 2 - Risk and Criterion 3 - Key Species (see Report on Species and Habitats Review p131-132 for full details). The adoption of ponds into the BAP corresponds with existing Annex 1 habitats under the EC Habitats Directive (92/43/EEC) such as; H3170 Mediterranean temporary ponds, H3130 Oligotrophic to Mesotrophic standing waters with vegetation of *Littorelletea uniflorae* and/or *Isoeto-Nanjuncetea*, H3140 Hard Oligo-Mesotrophic waters with benthic vegetation of *Chara* spp and H3160 Natural Dystrophic lakes and ponds.

The BAP states the UK has clear international obligations to conserve part of the habitat and associated species, under both the EC Habitats Directive and Water Framework Directives. Ponds support many species of conservation importance including an exceptional number (at least 65) of UK BAP priority species, six of which are listed in Annex 2 of the EC Habitats Directive. Ponds are also outlined at being under substantial risk as they are poorly represented in the SSSI site series and subject to serious degradation through enrichment, diffuse pollution and drainage to name just a few. Turnover of ponds still remains high, following the long term loss of pond numbers; this has only been partly compensated for in habitat creation.

UK BAP priority pond habitats are defined as “permanent and seasonal standing water bodies up to 20,000m^2 in extent, which meet one or more of the following criteria”;

1) Habitats of International Importance; ponds that meet criteria under Annex 1 of the Habitats Directive.

2) Species of high conservation importance: ponds supporting Red Data Book species, UK BAP species, protected species under the Wildlife and Countryside Act Schedule 5 and 8, Habitats Directive Annex 2 species, a Nationally Scarce wetland plant species, or three Nationally Scarce aquatic invertebrates.

3) Exceptional assemblages of key biotic groups: ponds supporting exceptional populations of key species based on; i) criteria specified in guidelines for the selection biological SSSI’s (amphibians and dragonflies); ii) exceptionally rich sites for plants and invertebrates (>30 wetland plant species or >50 aquatic macroinvertebrate species.

4) Ponds of high ecological quality: ponds classified in the top PSYM (Predictive System for Multimetrics) category “high” for ecological quality (>75%).

5) Other important ponds: individual or groups of ponds with limited geographical distribution that are recognised as important because of their age, rarity of type or landscape context e.g. dune slack ponds, Machair ponds and Pingo ponds.

3.4 Common Agricultural Policy (CAP)

The Common Agricultural Policy (CAP) is a system of agricultural subsidies and programmes covering agriculture, environmental measures and rural development. The potential to protect ponds and other water bodies through the use of buffer strips are incorporated into CAP schemes. However, there is currently no provision encouraging the construction of small ponds and wetlands. Other potential greening options include hedgerow restoration and the planting of nitrogen fixing or cover crops. Initially this has been through Environmental Stewardship schemes that subsidise farmers for good stewardship and management practices that improve the quality of the environment.

Currently the creation of buffer strips falls under 'greening' rules, specifically Ecological Focus Areas (EFAs), under farm Basic Payment Scheme (BPS). The rules state that if a farmer has more than 15 hectares of arable land, they will need to incorporate EFAs on their land, unless they qualify for exemption. EFAs are areas or features that the EU has decided are beneficial for the climate and the environment. Farms requiring EFAs must ensure that such areas or features are equivalent to a minimum of 5% of total arable land declared under the (BPS) application. Of the total agricultural land coverage in the UK (17.2 million hectares; Defra, 2012), arable land comprises about 4.75 million hectares. If all of this were to be incorporated into BPS schemes, this would equate to 236,500 hectares of EFAs. This highlights clear potential for a substantial number of ponds to be constructed under this policy mechanism, which would enhance biodiversity and functionality across agricultural landscapes.

4.0 Ecosystem Services

Ponds can contribute to sustainable solutions for a number of key issues, such as water management and climate change. They provide a wealth of valuable services, resulting from numerous physical-biological interactions, four of which stand out as having global significance and are as follows; biodiversity support, water quality improvement, flood abatement and carbon management (Céréghino et al., 2013; Boix et al., 2012; Williams et al., 2010; Downing, 2010; Zedler, 2005). Many of these processes occur simultaneously, providing a whole suite of valuable services. However, management for specific services is often at the detriment to others e.g. dredging of ponds to maintain suitable habitat for valuable open water/submerged macrophyte species, like *Chara*, essentially removes carbon stored within the system (Mitsch and Gosselink, 2000; discussions with Natural England reserve management team on the Lizard Peninsula SW England and SEFS conference delegates involved in the management of natural and aquaculture ponds). Understanding the interactions between such services is crucial, if we are to employ these features as ecologically effective and economically efficient tools in the construction of sustainable landscapes.

The ecological functioning and role of ponds is still being established, but is gaining increasing recognition by the scientific community, land managers and policy makers. Authoritative advice based on sound scientific evidence is required to inform future management and conservation of small water bodies if we are to link science and practice (Céréghino et al., 2013).

4.1 National Ecosystem Assessment

The UK National Ecosystem Assessment (NEA) 2011 was the first analysis of the UK's natural environment in terms of benefits provided to society and the nations continuing prosperity. It stated:

> *"The natural world, its biodiversity and its ecosystems are critically important to our well being and economic prosperity, but are consistently undervalued in conventional economic analyses and decision making"*

The principal objective of the UK NEA was to produce an independent and peer-reviewed assessment, of the state and value of the UK's natural environment and ecosystem services. It also aims to: i) identify past change in service provision from the natural environment; ii) understand what may drive future changes; iii) promote better interdisciplinary cooperation between natural and social scientists. Informed policymaking is essential to ensure effective management of the environment and ecosystem services provided.

Data taken from the Countryside Survey (Williams et al., 2007), found that perform a host of provisioning, regulating, cultural and supporting ecosystem services (see Appendices table IX.1). The assessment outlined that ponds in Scotland were generally in a better state than those of England and Wales. In England 80% of ponds were found to be degraded and of poor or very poor quality, supporting only a third of the total number of wetland plants and around one fifth of expected uncommon plants. The assessment also suggested ponds experienced a marked decline in quality between 1998 and 2007 as plant species richness decreased by 20% and the proportion of ponds categorised as poor or very poor increased by 17%.

Protection of ponds in the UK was found to be largely dependent on management of the surrounding landscape as oppose to designation of specific statuses (Williams et al., 2010). Eight habitats that partly or wholly include ponds are listed in the EC Habitats Directive, which also includes a range of Annex 2 listed species. Other than ponds and ditches designated under larger areas, Special Areas for Conservation (SAC's) created by the directive, generally only include water bodies larger than the UK's average pond area (<0.4ha). The WFD also places overwhelming emphasis on standing waters of more than 50ha. Only the BAP has given specific emphasis on ponds, yet only focuses on those with high conservational importance or particular ecological characteristics (Williams et al., 2010).

4.1 Biodiversity Conservation

Small water bodies have long been popular with naturalists and the general public alike. As previously mentioned, there has been a long standing bias in our understanding of ponds and their ecological and biogeochemical value. Early work on ponds was described as "the activity of the amateur, who's humble pond hunting, if carried out systematically and carefully, may....contribute to science" (Clegg, 1952). It was not until the 1990's that the value and importance of these wildlife habitats was truly recognised and appreciated by freshwater scientists.

Ponds and small wetlands are significant wildlife habitats, supporting populations of at least two-thirds of Britain's freshwater plant and animal species. At a national level they support 80 Biodiversity Action plan priority species, almost twice as many as lakes, and around 15% more than streams and rivers (Williams et al., 2010; Webb, 2008). Studies comparing species richness and value of ponds, against that of other freshwater ecosystems have yielded interesting results. The alpha (α) diversity of individual sites was found to be greater in rivers and streams. However, at a regional level they have demonstrated substantial gamma (γ) diversity, supporting more species, more unique

species and more scarce species, than other water bodies, due to the distinct heterogeneity observed between similar and different pond types (Céréghino et al., 2013; Boix et al., 2012; Jeffries, 2008; Davies et al., 2008; Williams et al., 2004).

A study of ponds in the UK, found that 75% of ponds sampled supported uncommon, scarce or Red Data Book species (Nicolet et al., 2004). Studies have also demonstrated substantial variation in species richness between pond types, with richest sites supporting similar numbers of taxa to good river sections and the poorest sites being amongst the most destitute of all water bodies. This highlights the susceptibility of ponds to degradation and the importance of considering them as conservational features in catchment management policies (Williams et al., 2004).

4.2 Water Quality Improvement

The ability of ponds and wetlands to be utilised in the removal of a number of diffuse pollutants such as nitrogen, phosphorus, suspended sediment and other contaminants, from surface waters, is well established (Tournebize et al., 2015; Mitsch et al., 2014; Vymazal, 2014; Zedler, 2005). Ponds strategically positioned to intercept water from drainage systems, have demonstrated the ability to significantly reduce nutrient loads of receiving waters, through sedimentation, denitrification and uptake from vegetation (Céréghino et al., 2013; Steidl et al., 2008). At a site scale, small bands of vegetated wetland, as little as 4m wide, constructed adjacent to streams can remove around 85% to 90% of Nitrates (NO_3^-), Phosphorus (P) and suspended sediments, carried in run-off (Zedler, 2005). Studies have demonstrated that ponds implemented for such services may also benefit local biodiversity (Becerra-Jurado et al., 2012; Herrman, 2012).

4.3 Flood Abatement

Ponds are becoming increasingly utilised in flood abatement schemes, such as Sustainable Urban Drainage Systems (SuDs) (Woods-Ballard et al., 2007). They can also be utilised in rural environments. Strategically placed pond networks hold water back at source, recharge aquifers and reduce volumes of water generated before they become a problem. Recent modelling studies have shown that by installing around 10,000m^3 of storage per km^2, roughly the size of ten intermediate sized ponds, it is possible to capture the majority of runoff produced from a typical heavy rainfall event for that km^2, significantly reducing water loss (Biggs, 2007; Quinn, 2007).

4.4 Climate Regulation

One of the less well-understood ecosystem services, provided by ponds and wetlands, is carbon sequestration and storage. Previous misconceptions by limnologists, that small aquatic systems are irrelevant in global biogeochemical cycling, mean that the scientific community is only recently exploring the true potential of these systems (Mitsch et al., 2013; 2014; Kayranli et al., 2011). Their absence in audits of biogeochemical cycling is most obvious in global carbon analyses. Recent audits have aimed to integrate the contribution of larger water bodies such as lakes and reservoirs to carbon cycling processes (e.g. burial and emission). However, the contribution of small water bodies to the carbon cycle has yet to be integrated. As studies increase in this area, so to do estimates of their global coverage and our understanding of the disproportionate intensity of carbon cycling.

"If we decide to take up the challenge of managing the earth's surface carbon cycle as a way of mitigating anthropogenic carbon emissions, we cannot ignore the contribution of inland water any longer" Battin 2009

Land based carbon mitigation strategies are considered an important and sustainable route towards climate stabilisation (Lamb et al., 2016; Canadell & Schulze, 2014). Sustainable strategies must be driven by integrated land management schemes, which take into account; limited quantities of land, and growing demands for food, wood products, energy, climate mitigation and biodiversity conservation (Canadell & Schulze, 2014).

If we are to understand the role of these systems in climate regulation and carbon cycling, we need to examine the two major factors governing this; i) their distribution and coverage at regional, national and global scales and ii) carbon cycling, particularly rates of burial and emissions. These topics will be discussed in more detail over the next several sections.

5.0 Global Coverage and Distribution of Inland Waters

5.1 Long term Misconceptions and Gross Underestimates

The important question of “how many lakes are there on Earth, and how big are they?” Remains one of the most critically unanswered questions in understanding biogeochemical cycling in inland waters (Seekall, 2013). Growing evidence supports the theory that small water bodies play a significant role in such cycles; perhaps this question should be re-addressed to include smaller water bodies not included under the definition of a “lake”.

Early catalogues of the global coverage and distribution of inland waters were first produced by Halbfaß (1914) and Thienemann (1925). Thienemann (1925) estimated around 2.5 million km^2, ~1.8% of the land surface, is covered with lakes and ponds, and that this area is dominated by a few very large lakes (Downing et al., 2009; Downing et al., 2006). This concept remained fundamentally unchallenged for almost a century, with estimates ranging from 2 - 2.8 million $km^{2,}$ or 1.3 - 1.8% of the earth’s continental area (Kalf, 2002; Schuiling 1977; Herdendorf, 1984; Meybeck 1995). However, Wetzel (1990) suggested that there are significantly more small lakes and ponds, and that these systems potentially dominate global freshwater surface area (Downing, 2010; Downing et al., 2006).

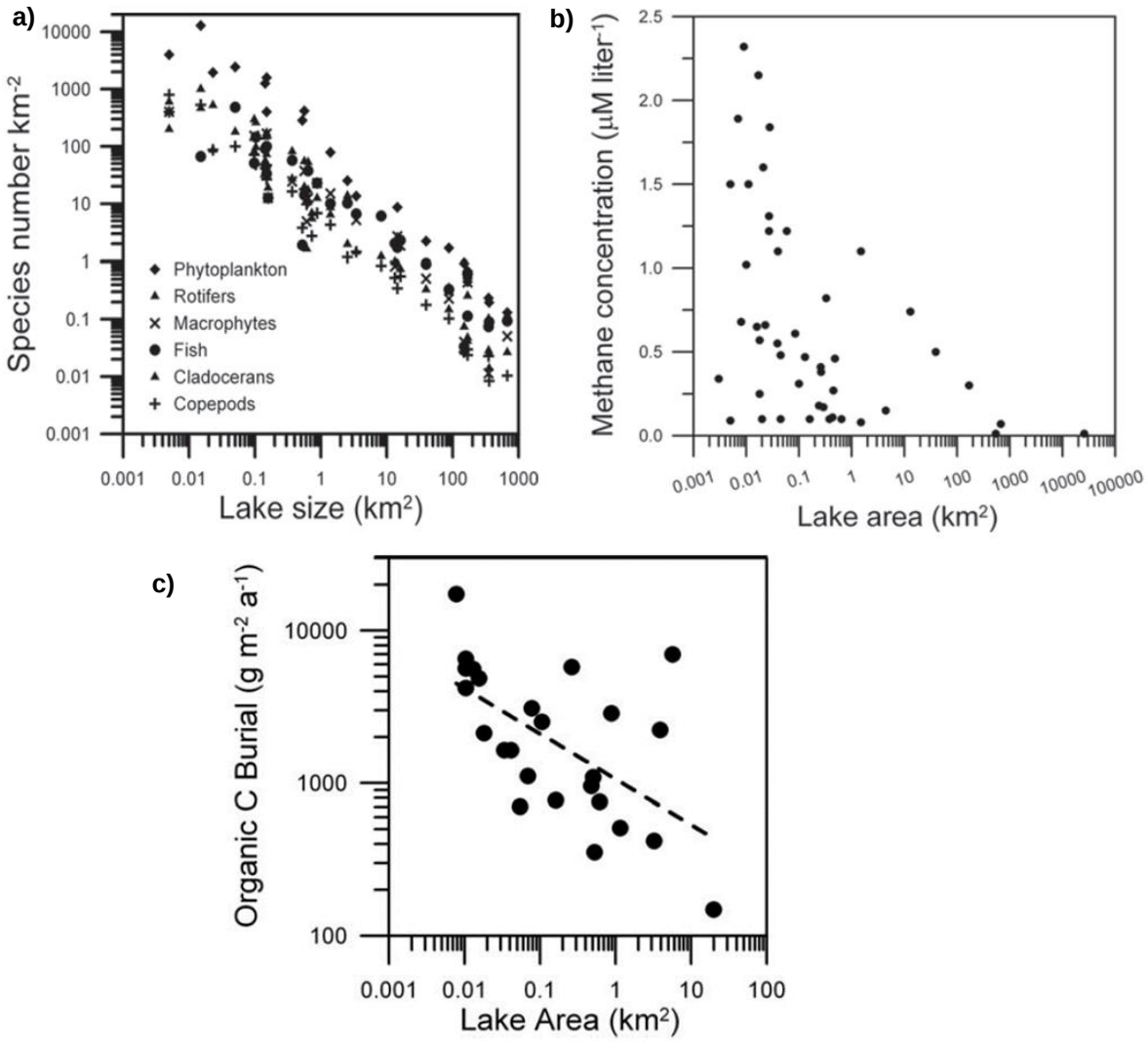
Recent studies have demonstrated that, the global coverage and distribution of global inland waters has been underestimated (Downing, 2010). The number and area covered by smaller water bodies in particular, has been subject to the largest degree of error (Verpoorter et al., 2014; Seekall and Pace, 2013). Contrary to popular misconception, the possibility that the coverage and distribution of smaller water bodies could be equal to that of larger lakes is fast becoming realised as interest and technological advances in this area of research grow.

5.2 Current Estimates

If we are to comprehensively understand the role of ponds in the carbon cycle, accurate estimates of their global coverage need to be produced. Of equal importance, is obtaining accurate estimates of the size distribution of water bodies within this overall coverage. Ecosystem functions and rates of aquatic processes are often determined by water body size. Disproportionate relationships are often observed between lake size, species richness, methane concentrations and carbon burial see figure I.1 (Downing, 2010; Downing et al., 2008). Smaller water bodies are more productive per unit area

than larger aquatic systems; their global coverage need only be equal to that of large lakes in order for them to dominate inland aquatic processing.

Figure I.1. a) Species-richness per unit area of various aquatic taxa in lakes of different sizes (Data from Dodson et al., 2000) taken from Downing 2008; b) Methane concentrations in lakes from around the world related to lake size. Data from Bastviken et al., (2004). c) Sediment organic carbon (OC) burial in impoundments measured in this study. Dashed line is the least squares regression between carbon burial and lake size (r^2=0.35, n=25,p=0.002). Taken from Downing et al.,2008.



Current estimates of the coverage and distribution of inland waters vary considerably and there has been fierce debate in recent decades over which methods provide the most accurate representation. Variability is largely dependent upon the resolution of satellite imagery, alongside specific remote sensing methods and the scales of maps and images used (Gala & Meleese, 2012; Baldwin & de Maynadier, 2009; Abedini et al., 2006). Discrepancies between remote sensing studies are most apparent in the

minimum size threshold used by individual studies, the majority of which are much greater than the surface area of small water bodies; >1km^2 (Lehner & Doll, 2004), 0.4 - 1.2 ha (Pitt et al., 2011), and 0.5 – 1 ha (Jeffries, 2015; Jones et al., 2009).

Lehner & Doll (2004) utilised a Global Lakes and Wetlands Database (GLWD) in order to produce estimates on their global coverage and size distribution. It was estimated that lakes cover around 3.2 million km^2, 2.4% of total global land surface (excluding Greenland) and that wetlands cover around 8-10 million km^2 or 6.2 - 7.6% of global land area. Results from this study indicated that lake size distribution followed power law patterns observed in previous research (Birkett and Mason, 1995; Meyback, 1995; Wetzel, 1990; Rapley et al., 1987).

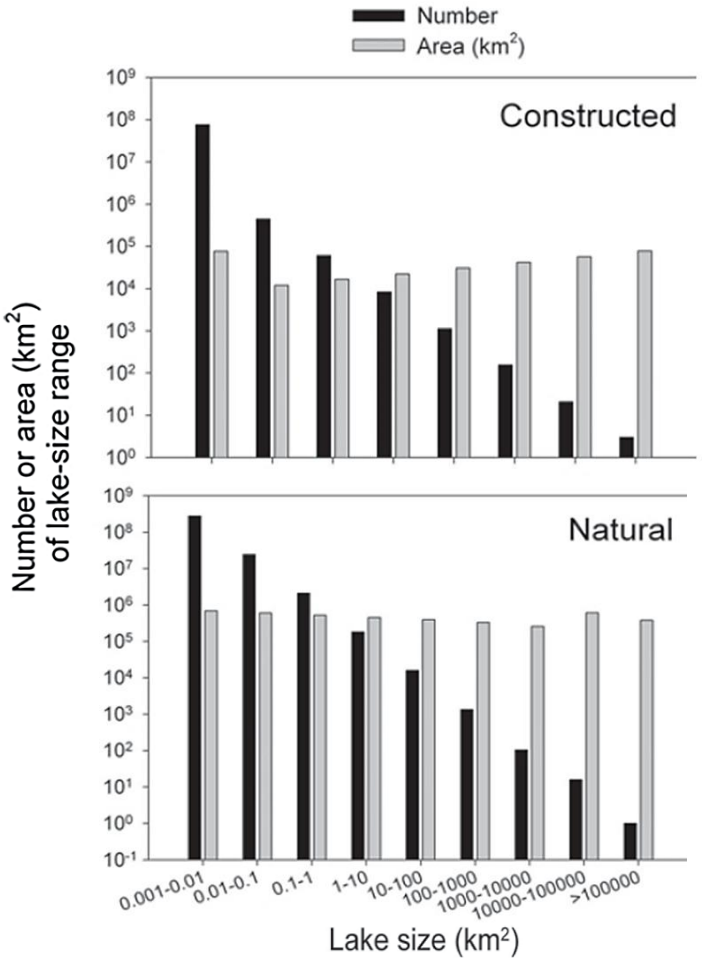
Downing (2006) utilised the Pareto Distribution, a power law probability model to estimate the global abundance and size distribution of lakes, ponds and impoundments down to 0.001km^2 (see figure I.2). The Pareto distribution estimated that the area of surface water bodies comprised of around 304 million lakes, covering 4.2million km^2, and that millions of water bodies smaller than 1 km^2 dominate this area. The study estimated at the lowest size category 0.001 km^2 there were around 277,400,000 lakes covering an area of around 692,600 km^2. Factoring in estimates of global impoundments at around >77,000 km^2, it was predicted that around 4.6 million km^2 or >3% of the earth's continental land surface is covered by water, almost twice that of previous estimates.

Downing (2010) attempted to use the Pareto Distribution to estimate the number of ponds in the size range 0.0001 – 0.001 km^2 (100-1000 m^2), to be around 3.2 x 10^9. The surface area of these water bodies was estimated to be around 0.8 billion km^2, though given the earth's surface area is 0.149 billion km^2, it would appear there is clear inaccuracy in these estimates suggesting a tendency for the Pareto Distribution to overestimate categories in the lower thresholds of the distribution (Jeffries, 2015). Recent modelling studies support the theory that estimates produced by power law probabilities, have the tendency to overestimate the abundance and coverage of small water bodies (Seekall et al., 2013; Mc Donald, 2012; Seekall & Pace, 2011).

A comprehensive analysis using high resolution satellite imagery was used by Verpoorter et al. (2014), in order to produce a Global Water Bodies database, comprising all lakes greater than 0.002 km^2 (GLOWABO). The study estimated that around 117 million lakes cover an area of around 5x10^6 km^2, roughly 3.7% of the earth's non glaciated land area, and that large and intermediate lakes dominate overall surface area. Lakes in the smallest size threshold 0.002 to 0.01 km^2 were the most

numerous at around 90 million, covering around 0.227% of the continental surface, whilst the 27 million lakes larger than 0.01 km^2 covered around 4.76 x 10^6 km^2. However, it was acknowledged that the smallest lakes comprise a large proportion of the global continental interface between land and freshwater, some of the most productive environments in terms of biodiversity and biogeochemical cycling (Downing, 2010; Bastviken, 2004; Wetzel, 1992). The importance of small water bodies as major contributors to biodiversity and biogeochemical cycling therefore stems from the higher productivity observed per unit area, as oppose to a dominance of overall lake surface area (Verpoorter et al., 2014; Seekall et al., 2013).

Figure I.2: Global size distributions of numbers and land area covered by natural and constructed lakes taken from Downing 2010.



Global inventories based on remote sensing and satellite imagery typically focus on waterbodies >0.001km^2. The number of water bodies <0.001 km^2 is likely numerous with small water bodies between 1m^2 and 1000 m^2 fairly common place throughout rural and urban environments. Estimates likely discount and underestimate the abundance of these features, due to natural temporal changes, such as seasonal

drying and succession (Soti et al., 2010). Accurate documentation on the numbers and size distribution of ponds, are generally obtained from recurrent ground surveys that identify seasonal features and ponds where ground visibility of aerial images is difficult, such as woodlands (Jeffries, 2015; Jeffries; 2012; Pitt et al., 2011; Calhoun et al., 2003).

5.3 Constraints

5.3.1 Pond loss and turnover

Pond loss and turnover is a significant factor compounding estimates of the distribution and coverage of these systems. Significant factors driving pond loss are often the result of either direct or indirect anthropogenic activity. Direct removal was common place in the early 20th C, as the usefulness of ponds had declined and traditional uses such as flax retting, horse washing after ploughing and the swelling of wooden wheels and barrels became redundant (Fairchild et al., 2013; Williams et al., 2010). Indirect removals from land draining were also commonplace, particularly affecting the hydrological regime of seasonal ponds and wetland areas.

At a national level it is estimated that pond numbers in England and Wales dropped by around 75% during the 20th century to around 200,000 by the 1980's (Biggs et al., 2005; Barr et al., 1994; Rackham 1986). At a regional scale, of the 41 564 small water bodies identified on ordnance survey maps of Cheshire in ~1870, 61% had disappeared by the early 1990's (Boothby & Hull, 1997). Latest national estimates suggest there were around 478,000 ponds throughout Great Britain in 2007, suggesting a high turnover, and general increase between 1998 and 2007, with 18,000 ponds lost and 70,600 ponds created (Williams et al., 2010). Increases are associated with the construction of ponds for leisure purposes as commercial fisheries, wildfowl hunting and on golf courses, alongside creation for education purposes and habitat improvement schemes (Williams et al., 2010). However, this survey failed to document features <25 m^2 in size, if these features were to be included estimates would likely be substantially more.

The dynamic nature of pond turnover throughout regional landscapes means snapshot surveys via satellite imagery or ground surveys may not provide an accurate representation of pond numbers and extent. Jeffries (2012) used a detailed map based audit of pond numbers in southeast Northumberland, recorded throughout several time intervals from the mid-19th century to 2008. The study found that from an original stock of 222 ponds mid-19th century, numbers had increased to 257 in 2008, though only 23 of the original ponds remained with substantial losses and gains throughout all map

survey dates, similar to other studies (e.g. Fairchild et al., 2013). The use of historical and current maps for documenting pond numbers is limited as seasonal and smaller aquatic features less than 4m^2 often go unrecorded (Jeffries, 2012).

Localised ground surveying has demonstrated that ponds exhibit substantial annual and seasonal changes in the number and wetted area of ponds, particularly in response to climatic variation and extreme weather events. Jeffries (2015) found the response of ponds to rainfall events was mediated by the surrounding land use, suggesting complex dynamics in the relationship between different pond types, land use and climate variability, which complicates attempts to quantify their abundance and coverage (Jeffries, 2015).

Understanding the number and extent of small water bodies is critical if we are to upscale any estimates of their biogeochemical cycling. However, there are clear barriers in our ability to accurately quantify this data, particularly the response of these systems to changing land use and rainfall patterns. Wetting and drying events lead to complex biogeochemical cycling in temporary ponds. The response of these variables to climatic variability is equally as important to understand (Reverey et al., 2016; Sahuquillo et al., 2012). Clearly more work needs to be carried out in this area if we are to produce any global models, but for now smaller scale ground surveys can provide invaluable information on intrinsic interactions between small aquatic systems, biogeochemical cycling and their response to local climate.

5.3.2 Succession

Another primary factor resulting in the natural loss of small water bodies is through ecological succession; the ‘natural’ lifecycle of a pond. Succession in ponds is the process by which they form or are constructed, eventually transitioning into semi aquatic, marshy environments, which potentially become completely terrestrialised. Formation may be through direct or indirect processes and may either be manmade (e.g. farm ponds, conservational ponds) or natural (e.g. beaver ponds, depressions in sand dunes, fallen trees, eroded landscapes). Although every pond is unique, they generally follow a similar trend in succession, which can be characterised as follows (see figure I.3 for conceptual model):

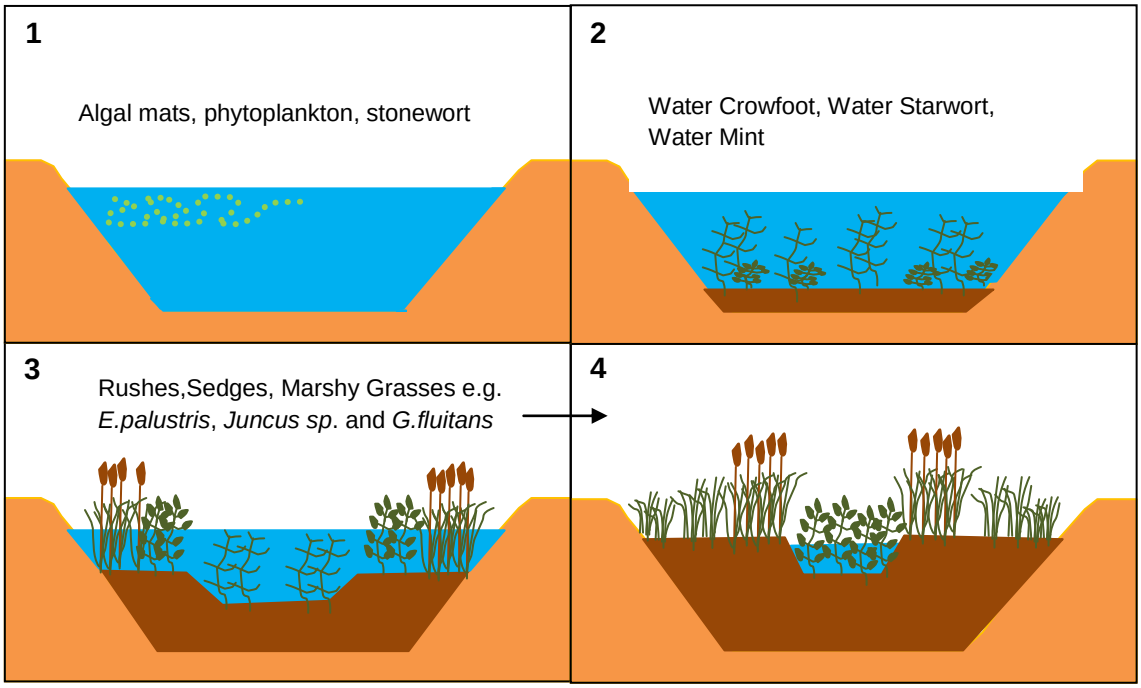
1) Early Succession: the pond is constructed or forms naturally. Water collects and remains permanent or may fluctuate with seasonal rainfall. Early pioneer species may establish, though productivity is likely to be limited to phytoplankton, filamentous algae or other algal-based species such as *Chara*, depending on nutrient levels. The bottom substrate is bare.

2) Mid Succession: macrophytes begin to establish in the pond. They may be submerged or emergent depending on the development of vegetation communities within the individual ponds. Seasonal growth and decay of vegetation results in the formation of sediment layers at the bottom of the pond. The majority of the pond surface is open water.

3) Late Succession: the accumulation of sediment promotes the establishment and emergent rooted species, and semi aquatic species on the fringes of the pond. Open water areas become sparse and the water column is substantially reduced.

4) Terrestrialisation: the pond appears more marsh like in character. Open water areas may no longer be visible as sediment levels and mats of vegetation breach the water level. These systems may dry out somewhat and can support terrestrial plant species. Alternatively, they may remain a waterlogged area and support a more bog/marsh based vegetation communities.

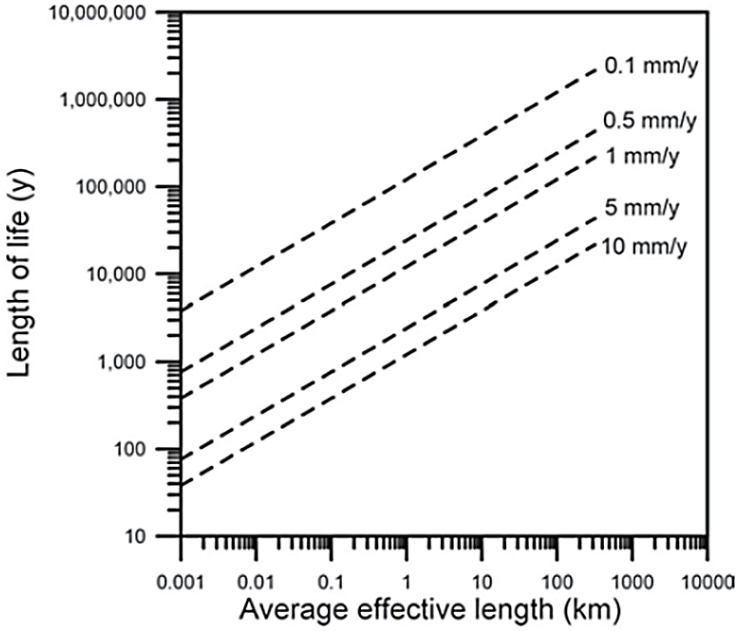
Figure I.3: Conceptual model showing the four general phases of pond succession: (1) Early Succession, (2) Mid-Succession, (3) Late Succession (4) Terrestrialisation.



The small size of these features mean that highly eutrophic systems, or those receiving stream inputs or large amounts of terrestrial matter (e.g. Forest ponds) may only last over a couple of decades prior to filling and succession see figure I.4 (Downing, 2010). However, less productive ponds can persist over much longer time frames such as East Anglian Pingo Ponds in Southeast UK, created at the end of the last ice age some

11,000 years ago (Clay, 2015). Natural pond succession processes are strongly influenced by anthropogenic activity, nutrient enrichment via agricultural run-off can speed up this natural process of succession via eutrophication. Dredging may be carried out to remove built up sediments restoring areas of open water within the pond.

Figure I.4: Potential lifetime of aquatic systems from a range of sizes. Taken from Downing 2010. Dashed lines indicate rates of infill.



Pond succession is equally important for the quantification of carbon fluxes and dynamics within these systems. Though little is known about physicochemical dynamics as ponds go through various stages of succession (Angéliebert et al., 2004) early succession ponds are generally characterised by algal species (e.g. Phytoplankton and Filamentous algae) or pioneer plant species (e.g. *Chara vulgaris*) (Fleury and Perrin, 2004). Although productivity might be high, bare substrate indicates almost complete remineralisation of organic matter, and that carbon storage at this stage is negligible. As succession occurs and rooted plants begin to establish, seasonal growth and decay results in accumulation of sediment at the pond bottom. This sediment is often rich in organic carbon amongst other nutrients. This indicates that carbon storage and burial is not uniform throughout the ponds lifespan, there is a threshold point at which accumulation and storage starts to become significant. It is therefore plausible that current stocks and accumulation rates for ponds globally may be a substantial component of the global carbon cycle.

Pond succession not only complicates the estimation of the abundance and coverage of small water bodies, it also plays an important role in determining how much carbon

is stored in the pond, and how much carbon that pond accumulates in a given year. Research linking together these important variables is required if accurate up-scaled models are to be produced for regional, national and global carbon audits on small water bodies.

5.3.3 Pond Hydro-period

Another important factor compounding efforts to accurately quantify the global coverage and distribution of small water bodies is hydro-period. The hydro-period of individual ponds, i.e., the period sediment stays inundated, stems from the net water balance, comprised of inputs (i.e. precipitation, snow melt, interflow, shallow groundwater discharge, surface run-off), outputs (i.e. evapotranspiration, lateral subsurface flow, shallow groundwater recharge) and pond morphology (i.e. surface – volume ratio) (Reverey et al., 2016; Dimitriou et al., 2009). Dry-wet cycles can be very erratic, responding dynamically to local temperature and rainfall (Reverey et al., 2016).

As previously mentioned, many small ponds are temporary in nature, which has led to their omission in regional map based audits (Jeffries, 2012; Williams et al., 2010). The fundamental nature of temporary wetlands, the very characteristics that support a wealth of biodiversity, such as small size and dynamic behaviour, complicates survey efforts (Jeffries, 2016). It has also been stated, that ponds go unprotected because they are undetected (Baldwin & Maynadier, 2009).

Contention over the contribution of small water bodies to overall numbers and coverage of inland waters has highlighted a need for accurate ground surveys to record temporary wetlands. Field surveys, such as the UK Countryside Surveys carried out in 1996 and 1997, have highlighted the widespread abundance of seasonal ponds in the UK (Williams et al., 2010). Their ecological importance has been highlighted in previous sections, but less well understood is their susceptibility to future climate change. Pond numbers and size vary with climate, both seasonally and in response to changing climate regimes and extreme events (Jones, 2013). The area, density and clustering of ponds has a pronounced effect on species richness at the landscape scale (Oertli et al., 2002), the same will also be true for carbon fluxes. Variability between years and changes associated with local climate are important but remain under-researched (Jeffries, 2016).

Jeffries (2016) is one of the first detailed field surveys, quantifying pond numbers and size over a sustained 3 year time period. Results demonstrated that the number and size of wetted ponds varied markedly between years and seasons, and displayed a strong relationship with regional rainfall. Results also highlighted that different pond

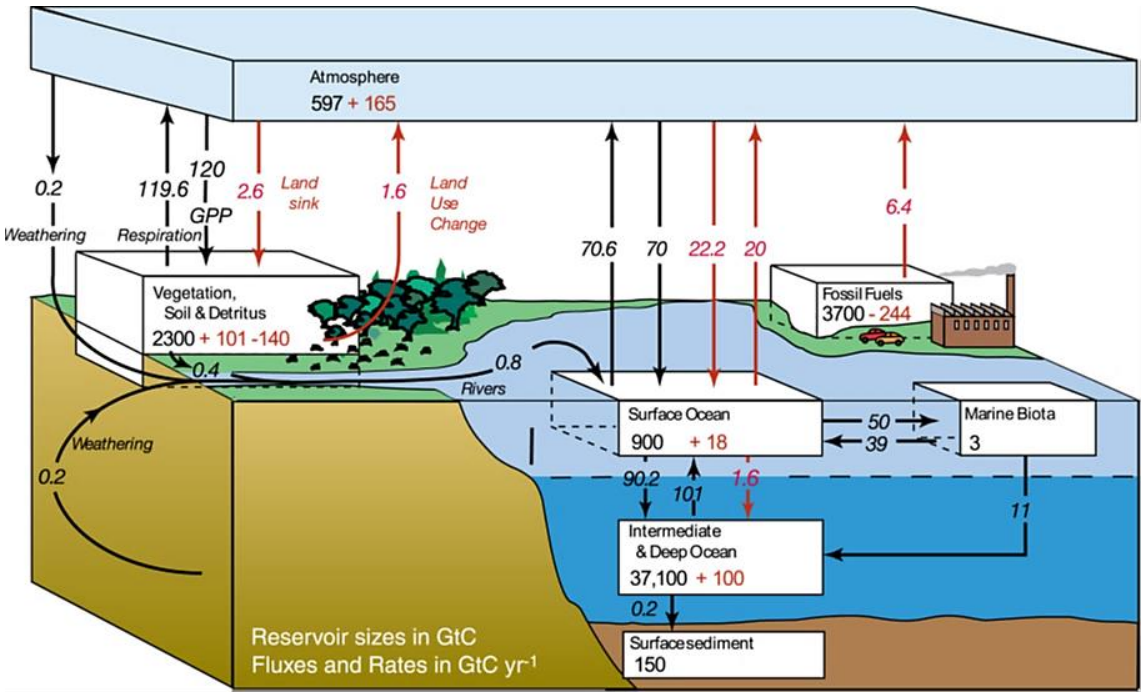
types and ponds surrounded by different land use, were affected by rainfall over different time scales. Given the marked variability observed within this survey it was suggested that rather than quantifying an absolute number of ponds within a landscape, surveys should attempt to quantify ranges of pond size and numbers, characteristic of the landscape type. The study concluded that the threat to temperate ponds from climate change is likely to be an intricate interplay between local climate, pond type and surrounding land use.

Emerging research has demonstrated that the hydrological behaviour of temporary aquatic features leads to complex biogeochemical cycling, particularly carbon fluxes (Reverey et al., 2016; Gilbert et al., 2016, Catalán et al., 2015). Quantifying the range of pond size and numbers within a landscape, alongside carbon fluxes in response to local climate, is critical to elucidating the role of these habitats in the carbon cycle. Carbon cycling within inland waters and ponds will be discussed in more detail in the following section.

6.0 Inland Waters and the Global Carbon Cycle

The global carbon cycle comprises of three interlinked components of the biosphere: terrestrial, oceanic and atmospheric. Terrestrial and oceanic components are biologically active, in that they can sequester or release carbon via gas fluxes with the atmosphere. These three components have formed the basis of many simplified models of the carbon cycle (Cole et al., 2007; IPCC, 2001), which although quite simplistic, were informative in the identification of major imbalances and clear knowledge gaps. Later models have been more comprehensive incorporating a number of sub compartments and processes to the biologically active components see figure I.5 (IPCC, 2007).

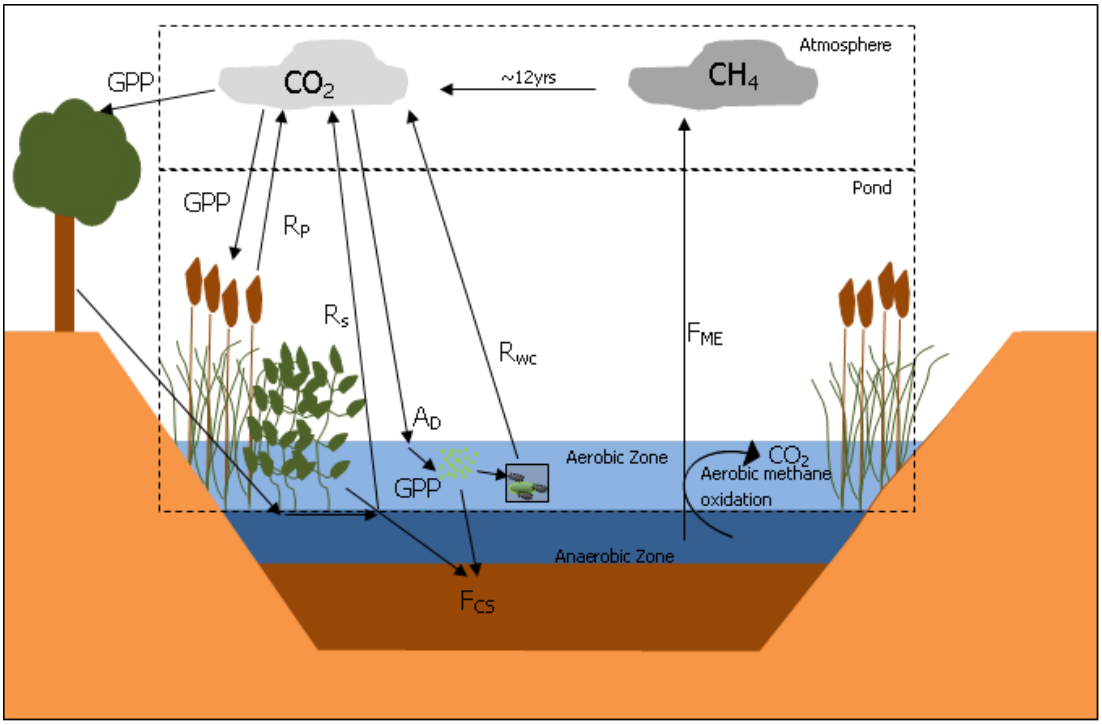
Figure I.5: The global carbon cycle for the 1990s, showing the main annual fluxes in GtC yr^{-1}: pre-industrial 'natural' fluxes in black and 'anthropogenic' fluxes in red (modified from Sarmiento and Gruber, 2006, with changes in pool sizes from Sabine et al., 2004a).



Natural ecosystems are of paramount importance to the carbon cycle as they currently offset ~4 Pg C yr^{-1} of anthropogenic carbon emissions (Raymond et al., 2013; LeQuéré et al., 2009). Research into the various sub components of the carbon cycle has led to their addition in more comprehensive audits (e.g. Canadell et al., 2014; Cramer et al., 2001). However, more research into unknown components is required if we are to comprehensively understand and predict future climate change.

Photosynthetic assimilation of atmospheric carbon is a major pathway in which carbon enters biologically active components of the carbon cycle. Also known as, Gross Primary Production (GPP), this carbon supports the growth and respiration of living organisms. Upon the death and decay of these organisms, carbon can undergo photo-oxidation or remineralisation by microbial communities, releasing carbon back into the atmosphere as CO_2 or CH_4, depending on environmental conditions. In productive environments not all carbon under goes respiration and evades remobilisation to the atmosphere. This carbon is known as Net Ecosystem Production (NEP) and is usually measured as units of carbon over time (e.g. g C m^{-2} yr^{-1}). Either it can be stored within the system as organic biomass, or it can be exported elsewhere via erosion and transportation. Determining whether a given ecosystem is a net sink or source of carbon, requires accurate quantification of rates of GPP, respiration, storage and export. Net sinks of carbon will have a positive NEP, whereas net sources will be negative. A full conceptual model of fluxes, storage and emission pathways in ponds can be seen in figure I.6.

Figure I.6: Conceptual model of carbon fluxes in ponds. F_{cs} carbon sequestration, F_{me} Methane emission; $_{AD}$ atmospheric diffusion; GPP gross primary productivity; R_P Plant respiration; R_S soil respiration: R_{WC} water column respiration.



Inland waters, excluding large wetlands, have long been overlooked as playing a significant role in the global carbon cycle. Largely due to the belief, they were inactive

in terms of carbon processing, coupled with the relatively small proportion of the earth's surface they cover. Current research indicates that this is far from true. They occupy a much larger area globally, processing substantial amounts of carbon. However, there are conflicting opinions whether these systems are net sinks or sources. Inputs from both allocthonous (terrestrial) and autochthonous (aquatic) organic matter (OM) mean that these systems are often classified as net heterotrophic (Raymond, 2013), processing a combination of internally and externally derived carbon. Contrary to this, the accumulation of sediment and subsequent burial of organic carbon (OC) results in net storage within the system. The fate of OM in these systems is ultimately governed by its chemical composition and molecular structure (Reverey et al., 2016). Determining whether these systems are net sinks or sources requires an understanding of factors impacting OC storage and remineralisation. General principles and key processes will be discussed over the following sections.

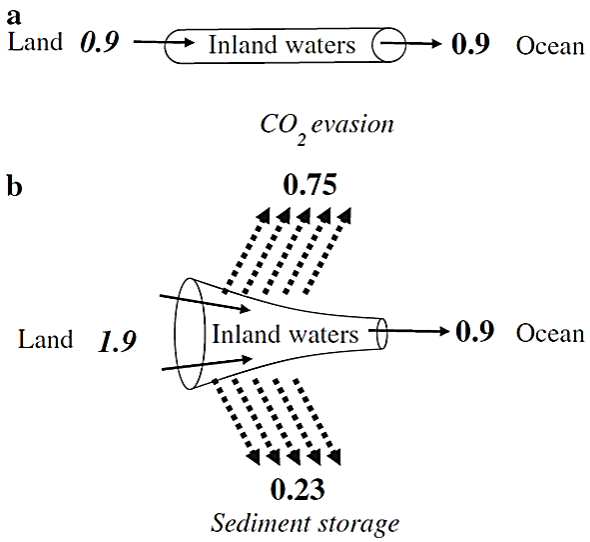
6.1 Net Sink?

A key concept in sub compartmental research of inland waters in the carbon cycle was the "riverine or neutral pipe". It was discovered that rivers deliver significant amounts of OM and inorganic carbon (IOC) from land to sea (Degens et al, 1991; Schlesinger and Melack, 1981). Lakes and ponds were rarely included, or were integrated with the riverine pipe, under the belief that OC was transported from, as opposed to processed by, these systems see figure I.7 (Cole et al., 2007).

Early research into OC burial in lake sediments estimated rates of around 30 to 70 Tg C yr^{-1} (Einsele et al., 2001; Dean & Gorham, 1998; Mulholland 7 Elwood, 1982), Estimates for impoundments were more substantial, at 150 to 220 Tg C yr^{-1} (Stallard, 1998; Mulholland & Elwood, 1982). These estimates were comparable to rates observed in ocean sediments (120-260 Tg C yr^{-1}; Duarte et al., 2004; Sundquist, 2003; Meybeck, 1993), and OC transported from rivers to the ocean (400 Tg C yr^{-1}; Meybeck, 1993). Although, estimates were small in comparison to the terrestrial sink (1000 to 4000 Tg C yr^{-1}; Randerson et al., 2002; Pacal et al., 2001; Schimel et al., 1995).

Further research proposed an "active pipe" model to replace the riverine and neutral pipe concepts. Conservative estimates suggested inland waters receive 1.9 Pg C yr^{-1} from the terrestrial landscape, 0.2 Pg C yr^{-1} is buried in aquatic sediments, at least 0.8 Pg C yr^{-1} is remobilised to the atmosphere, and 0.9 Pg C yr^{-1} delivered to the oceans see figure I.7b, in equal proportions of IOC and OC (Cole et al., 2007).

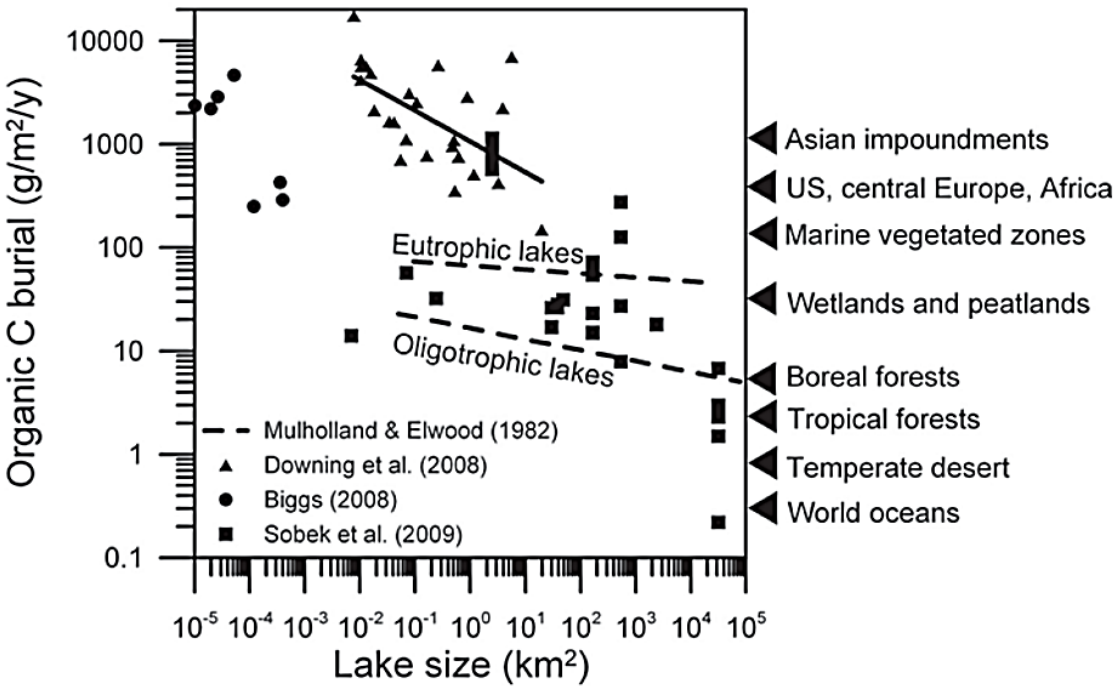
Figure I.7: Conceptual model outlining (a) the neutral pipe model and (b) the active pipe model for inland aquatic processing of carbon. Taken from Cole et al., 2007.



Typical of the bias towards larger water bodies observed in limnological studies, initial estimates focused largely upon large lakes and reservoirs, with little consideration to small water bodies. Research into small agricultural impoundments suggests that small water bodies may actually bury significantly more OC than previously thought (Downing et al., 2008). Estimates of OC burial ranged from 148 – 17,000g C m^{-2} yr^{-1} with a median of 2122g C m^{-2} yr^{-1}, suggesting these systems were significantly more active in carbon cycling than previously thought. It was proposed large inputs of allocthonous OM, coupled with enhanced autochthonous OM result in high preservation levels due to sediment anoxia, promoting substantial burial rates of OC see figure I.8 (Downing et al., 2008). The acknowledgement of substantial autochthonous OM inputs represented an active carbon sink previously overlooked in limnological research.

Upon discovering these systems were processing around 1 Pg of carbon more than previously thought (Downing, 2010), further research attempted to integrate these systems into the global carbon cycle. Revised models were produced, based on up-scaled areal coverage, reported burial rates and the active pipe concept see figure I.9 (Battin et al., 2009). It was estimated that globally these systems bury around 0.6 Pg OC yr^{-1} and emit around 1.4 Pg OC yr^{-1}. Although, estimates were based on reservoirs and lakes, with little consideration of OC burial in small water bodies, such as ponds.

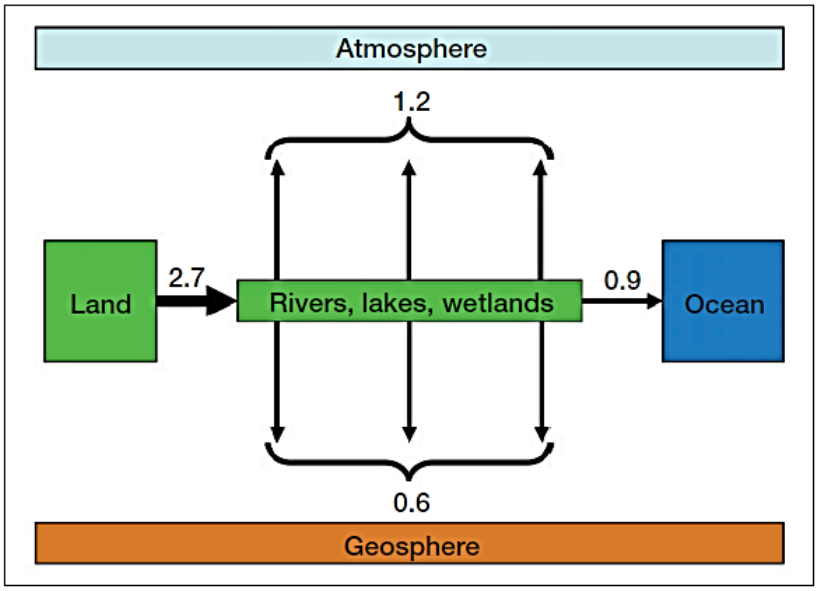
Figure I.8: Sediment organic carbon burial rates compared among types of aquatic and terrestrial ecosystems. Taken from Downing 2010.



Increasing interest in lake carbon cycling dynamics, led to research attempting to predict OC accumulation in lake sediments, which could then be up-scaled to regional levels. Key drivers identified so far include lake area (Ferland et al., 2012; Kortelainen et al., 2004), the lake dynamic ratio (√ lake area/ mean water depth) (Ferland et al., 2012) alongside other catchment features (Kortelainen et al., 2006; Kortelainen et al., 2004). However, uncertainties remain as to the burial efficiency of deposited OC material and proportion of OC that is permanently buried (Ferland et al., 2014).

OC burial efficiency represents the proportion of OM that escapes remineralisation and is permanently buried. Research has identified that burial efficiency is dependent on factors influencing OC degradation (Sobek et al., 2011; Sobek et al., 2009: Hartnett et al., 1998), such as temperature (Gudasz et al., 2010) and oxygen exposure (Fenner and Freeman, 2011; Sobek et al., 2009). The origin of OC has also been found to strongly influence burial efficiency, as autochthonous OM is preferentially degraded, promoting disproportionate accumulation of allocthonous material (Gudasz et al., 2012; Sobek et al., 2009). Studies also demonstrate that long term burial rates in lakes are identical to current centennial scale accumulation rates and conclude that degradation after a few decades is negligible (Ferland et al., 2014).

Figure I.9: Coupling of land, oceans and atmosphere by rivers, lakes and wetlands. All numbers are fluxes in units of Pg C yr^{-1} reported in Battin et al. (2009). Taken from Aufdenkampe 2011.



A key theme arising from Downing's (2008) study was the disproportionate intensity of OC burial observed with decreasing water body size (see figure I.8). This prompted further research into OC burial across small water bodies, especially given recently up scaled revisions of their global abundance and coverage. Compiling data for OC burial in a number of inland aquatic systems, Downing (2010) found these systems to bury OC orders of magnitude greater than the majority of other global systems, such as forests (0.7 - 12 g OC m^{-2} yr^{-1}), grasslands (2.2 g OC m^{-2} yr^{-1}) and agricultural land returning to grassland (1.6 - 110 g OC m^{-2} yr^{-1}). Substantial burial rates compensate for their relatively small global areal coverage, indicating their importance as an active environment within the biosphere, and the need for further research into the role of these systems in the global carbon cycle (Downing, 2010).

The role of ponds in carbon cycling, particularly OC burial, transport and emission is poorly understood in comparison to other inland waters (Tranvik et al., 2009; Cole et al., 2007; Dean & Gorham, 1998). As a result they are absent in the majority of major reports outlining the OC storage capabilities of global and national habitats, notably IPCC 2007, UNEP 2009 and Natural England (Alonso, 2012). Forests, grasslands and wetlands are included in such reports, however, inland waters are often excluded or focus on large lakes. In theory, ponds provide environmental conditions to support enhanced burial of OC, but the same also applies for emissions of CO_2 and CH_4. Understanding and quantifying processes within the ponds, is vital if we are to determine whether these small aquatic systems are net sources or sinks of carbon.

6.1.1 OC% and Burial

Gilbert et al. (2014) was one of the first studies to analyse variations in sediment OC across a range of small ponds. Results demonstrated that sediment OC varied considerably between pond types and was highest in permanent ponds with extensive natural vegetation (7.68 – 12.68%). Ponds in arable or pasture fields had much lower sediment OC (3.44 - 3.72%), and were more comparable to that of adjacent soils (3.13 - 3.8%). The study was one of the first to report burial rates in small ponds. Burial rates were on average 149 g OC m^2 yr^{-1} (108–173 g OC m^{-2} yr^{-1}). These values are some of the highest reported in literature and were elevated substantially above burial rates observed across a range of terrestrial and aquatic habitats, see table I.3.

Table I.3 Carbon burial in a range of environments. Data for lakes and impoundments are from *Mulholland and Elwood (1982),* data for terrestrial ecosystems including peatlands are from *Schlesinger* (1997), data for marine ecosystems are from *Duarte et al.* (2005), data from Aquaculture ponds taken from Boyd (2010), Data for Wetlands taken from Mitsch (2013), Data for small impoundments Pittman (2013), data from temperate reservoirs Sobek (2010). Data in red taken from this study. (Adapted from Downing et al. 2008).

Environment[a]	Mean OC Burial[b] rate (g m^{-2} yr^{-1})	Range
Agricultural Impoundments	2122	148 - 17,392
Temperate Reservoir	1113±482	536 - 1950
Impoundments (Asia)	980	20 - 3300
Impoundments (Central Europe)	465	14 - 1700
Impoundments (United States)	350	52 - 2000
Impoundments (Africa)	260	
Small Impoundments (Missouri)	236.75	183 - 279
Small Constructed Ponds	149	108-173
Aquaculture Ponds	148.9±90.3	28 - 333
Marine Vegetated Habitats	139	83 – 151
Wetlands	118	42 - 306
Small Mesotrophic Lakes	94	11 – 198
Abandoned Agricultural land, Returning to grassland	56	1.6 - 110
Mine Spoils Returning to Forest and Grassland	42	28 - 55
Peat lands	31	8 – 105
Marine Depositional Areas	31	17 – 45
Abandoned Agricultural Land Returning to Forest	30	21 - 55
Small Oligotrophic Lakes	27	3 – 128
Large Mesotrophic Lakes	18	10 – 30
Large Oligotrophic Lakes	6	2 – 9
Boreal Forest	4.9	0.8 – 11.7
Temperate Forests	4.2	0.7 – 12
Tropical forests	2.4	2.3 – 2.4
Temperate grassland	2.2	
Tundra	1.2	0.2 – 2.4
Temperate Desert	0.8	

This study concluded that substantial variation in OC% between different pond types and variations in OC burial across superficially similar ponds likely reflects their variable productivity, based on pond permanence, nutrient inputs, vegetation cover and trophic status. The heterogenous nature of pond ecological communities undoubtedly plays an important role in determining the magnitude of ecosystem functions and processes, such as OC burial. This poses significant implications when attempting to integrate results into global carbon budgets and when assessing their potential as features for effective carbon mitigation. Therefore elucidating key factors driving OC burial is crucial to comprehensively understanding carbon dynamics in these systems.

6.1.2 Organic carbon sources and storage

Aquatic and wetland environments provide optimum conditions for sequestration and the long-term storage of CO_2 (Mitsch et al., 2013). As previously mentioned, sources of OC in inland waters can be derived from allocthonous sources transported into the system, or autochthonous productivity. The origin and proportions of OC sources can strongly influence OC burial efficiency in lake sediments. Autochthonous material is preferentially degraded but also leads to increased sedimentation rates and preservation of allocthonous material (Reverey et al., 2016; Ferland et al., 2014). Higher inputs of allocthonous material have also been shown to affect the OC burial efficiency of sediments (Sobek et al., 2009).

Inputs of allocthonous material can come from a number of sources and are largely dependent on the catchment of the receiving water. Erosion of soil at the land water interface and in the catchment area during surface run-off is a substantial pathway through which material is delivered into inland waters and fluvial networks. Therefore vegetation cover and land use in the surrounding catchment plays a significant role in the proportion and types of OM delivered to inland waters (Nitsche et al., 2017; Reverey et al., 2016). A large number of inland waters are stream fed and subject to transport from fluvial networks. Slower and deeper water promotes suspended particulate matter to drop from the water column and accumulate in sediments, particularly in impounded waters and reservoirs. This likely accounts for the higher OC burial rates observed in these systems in comparison to natural lakes (Tranvik et al., 2009; Downing et al., 2008).

The relatively large land-water interface occupied by smaller water bodies in comparison to lakes and reservoirs renders them particularly susceptible to allocthonous inputs via erosion processes (Verpoorter et al., 2014; Downing, 2010). Many ponds rely on surface run-off from the surrounding catchment for hydrological

recharge, which can also deliver terrestrial OM to the pond. Stream fed ponds are particularly vulnerable to siltation and may require regular dredging to remove accumulated sediment. Ponds located near trees are also subject to large inputs of leaf matter, often at the detriment to the ponds biodiversity value (Biggs et al., 2005).

Many believe that inland waters are net heterotrophic as a result of allocthonous OM inputs (Raymond et al., 2013). This may be true for waters where inputs exceed autochthonous productivity. However, many ponds reveal substantial rates of productivity, particularly small shallow ponds and lakes. Theoretically, ponds support conditions for enhanced productivity and growth of vegetation, due to decreased dilution of nutrients and shallow conditions that promote enhanced photosynthetic activity (Downing, 2010). Limited proportions of allocthonous inputs in the majority of these systems compared to larger waters, means that autochthonous materials dominate inputs of OM, and may contribute to enhanced OC burial (Downing, 2010).

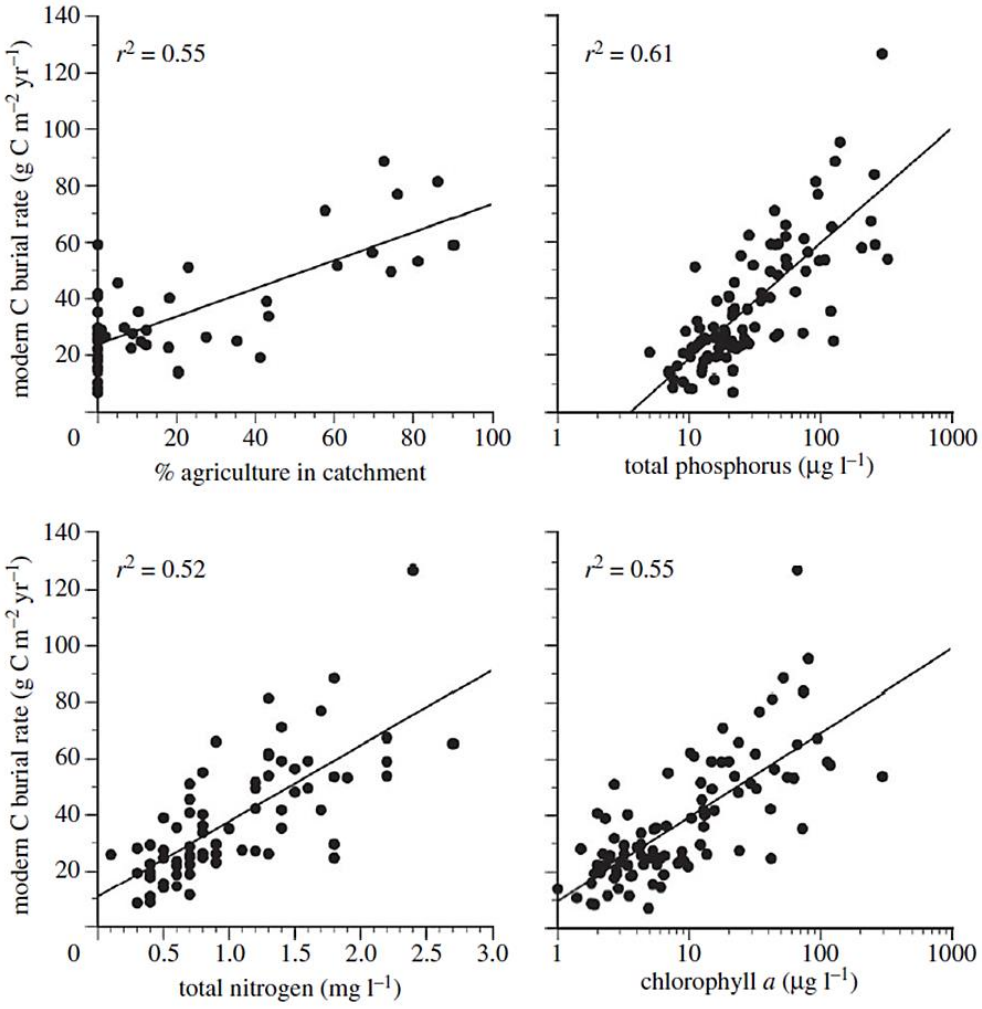
6.1.3 Productivity

Autochthonous productivity is established as a primary factor driving OC burial and species richness in aquatic systems (Downing, 2010; Downing et al., 2008). It is strongly linked to increased OC sedimentation rates (Ferland et al., 2014; Sobek et al., 2009; Cranwell, 1981; Ho and Meyers, 1994), although, this is largely dependent on watershed properties (Hakanson and Jansson, 1983). Productivity depends largely on light penetration, water clarity and nutrient availability, although thermal and nutrient stratification can also be a limiting factor in larger lakes. Productivity in lakes and reservoirs is often restricted to vegetation growth in shallow margins or phytoplankton in the photic zone, leading to large areas of unproductive open water (Downing, 2010). On the other hand smaller water bodies make up a large proportion of the global land lake interface, and these areas are known to be highly productive environments. These features are generally much shallower and vegetative growth can occur throughout the majority of the water area (Della Bella et al., 2007), accounting for the disproportionate species richness and carbon burial observed with decreasing lake size.

Smaller water volume promotes nutrient enrichment and availability, particularly in agricultural landscapes (see figure I.10) and can significantly alter aquatic thermodynamics, with ponds responding much more quickly to climatic changes in temperature than larger water bodies (Chaichana et al., 2011). Although vulnerable to freezing over in colder months, in direct sunlight shallow features can reach temperatures substantially above surrounding air temperatures, facilitating enhanced winter productivity (Personal observations throughout the study period).

Autochthonous productivity can also contribute to OC burial by altering sediment and sediment-water interface conditions. Large inputs of OM and sedimentation rates result in a biological oxygen demand at the sediment surface, significantly reducing rates of aerobic respiration and promoting sediment anoxia. Sediment anoxia supports anaerobic respiration of OM, which is generally much slower than the former, although, CH_4 is produced as a result and is more potent than CO_2 in its climatic warming effect.

Figure I.10: Relationships between contemporary C accumulation rates (g C m^{-2} yr^{-1}) and selected limnological variables [total phosphorus, total nitrogen and chlorophyll-a, n=97, 74 (after outliers being removed) and 96 samples respectively and percentage agricultural land-use in catchment (n=52)]. Taken from Anderson et al., 2013.



6.1.4 Preservation

Sediment anoxia often results in the formation of OC rich sediments and preservation of OC (Gelinas et al., 2001; Pederson & Calvert 1990; Demaison and Moore, 1980). Oxygen limited environments limit the rate at which heterotrophic bacteria can degrade

OM, and promote the burial of accumulated OC within sediments. High primary productivity was found to be a dominant factor driving sediment anoxia in some studies (Lee & Biggs, 2015; Pederson & Calvert 1990). Substantial burial rates of OC observed in eutrophic agricultural impoundments (Downing et al., 2008) have been associated with high inputs of eroded allocthonous material, alongside enhanced nutrient driven primary productivity. Large inputs of OM accumulating in sediments, exceeds the capacity of heterotrophic communities to degrade OM, creating a high biological oxygen demand in sediment layers and at the sediment water interface. Slower remineralisation rates under these anoxic conditions promote the preservation of OC, particularly allocthonous material due it recalcitrance and the preferential degradation of autochthonous OM (Reverey et al., 2016; Ferland, 2014; Downing et al., 2008).

A negative relationship was also found between oxygen exposure time and the burial efficiency of OC in both marine and lake sediments. Oxic conditions favour the remineralisation of OC to CO_2 (Sobek et al., 2009; Märki et al., 2006; Hedges et al., 1999; Hartnett et al., 1998). Small aquatic systems have a high OC burial efficiency in comparison to other aquatic environments. However, their small size renders them susceptible to seasonal changes in hydrological regime, exposing sediment layers. Sediments can quickly change from anoxic to oxic, increasing microbial activity and mineralisation rates of OC (Reverey et al., 2016; Catalán et al., 2014; Fromin et al., 2010). The intricate hydrological dynamics of these systems create complex dynamics in biogeochemical cycling, and a net sink of CO_2 may quickly become a net source, over short time scales (Gilbert et al., 2016). If we are to determine with certainty whether small water bodies are net sources or sinks, we must quantify and understand rates of CO_2 and CH_4 remobilisation, particularly in response to climatic variables (Catalán et al., 2014).

6.2 Re-mineralisation and Emission

Aquatic environments receive large amounts of OC, either that assimilated in the water column, or via transport from terrestrial environments. The fate of this OC is burial, transport or remobilisation to the atmosphere via mineralisation or photo-oxidation. Processes governing OC burial in inland waters have been discussed previously, but little has been mentioned about the mineralisation and remobilisation of carbon back to the atmosphere. Key processes driving OC remobilisation are microbial degradation of OM, and the subsequent movement of gas through the water column prior to release at the water-atmosphere interface.

Studies have indicated that the majority of OC mineralisation occurs within the first five years after deposition (Anderson et al., 2013; Galman et al., 2008). Other estimates for boreal lakes suggest this may occur over the first few decades, after which remineralisation is negligible (Ferland et al., 2014). Over this time period it is estimated that there is, on average, a 10% loss in OC within the upper layers of sediment, which Clow (2015) suggested would cause burial rates to be overestimated by ~6%, and that adjusting observed burial rates downward by 6% would likely account for mineralisation within recent sediments (Clow et al., 2015; Anderson et al., 2013).

6.2.1 Net source?

A number of studies have argued that global inland waters are a net source of carbon to the atmosphere. A range of CO_2 estimates for a number of aquatic environments in various equatorial zones can be viewed in Table I.4. Understanding rates of release of both CO_2 and CH_4 from aquatic systems is important if we are to fully understand their role in the carbon cycle, though complex dynamics between OM inputs and environmental conditions across spatial scales make up-scaling estimates difficult.

There are relatively few studies which have attempted to estimate global CO_2 emissions from inland waters. Early regional estimates suggested in the Amazon region alone, streams, rivers and wetlands were releasing around 0.5 Pg C yr^{-1} (Aufdenkampe et al., 2011). Preliminary global estimates of CO_2 emissions from inland waters suggested an efflux of around 1 Pg C yr^{-1} (Battin et al., 2009; Cole et al., 2007). More recent studies, which have integrated upscaled estimates of rivers and head water streams, suggest this figure may actually be ~2.1 Pg C yr^{-1} (Raymond et al., 2013). Raymond (2013) also suggested that proportions of emissions from streams and rivers were much higher (~1.8 Pg C yr^{-1}) than lakes and reservoirs (~0.32 Pg C yr^{-1}). Aufdenkampe (2011) in a more comprehensive report, estimated carbon emission values are around ~3.28 Pg C yr^{-1}, although wetlands were included in this report and contribute a much higher proportion (~2.08 Pg C yr^{-1}) than other inland waters. The inclusion of wetlands and riparian flood zones, adds significantly more global coverage, particularly in tropical areas, which are well known to emit higher rates of CO_2 (Bridgham et al., 2013; Gonzalez-Valencia et al., 2013; Aufdenkampe et al., 2011).

Fewer studies have focused on annual CH_4 emissions to the atmosphere from inland waters and wetlands. Current estimates for emissions from lakes are broad and in the range of 6 - 36 Tg C yr^{-1} (Bastviken et al., 2004). In comparison to CO_2 emission rates, CH_4 efflux is estimated to around 4% of that emitted as CO_2 (Cole et al., 2007). Comprehensive estimates of CO_2 and CH_4 efflux from inland waters are compounded

by relatively few studies carried out on water bodies in the smallest size categories, which greatly outnumber large lakes and reservoirs. It is likely that similar patterns in disproportionate rates of OC burial associated with decreasing lake size, might also be observed in rates of remineralisation due to the intensity of biogeochemical processes.

Table I.4: Estimates of CO_2 emissions from inland waters, for zones based on atmospheric circulation. Taken from Aufdenkampe et al., (2014).

Zone-Class	**Area of inland waters (1000s km^2)**	**pCO_2 (ppm)**	**Gas exchange velocity ($k_{600}$$^{-9}$ cm hr^{-1})**	**Areal outgassing (g C m^{-2} yr^{-1})**	**Zonal outgassing (Pg C yr^{-1})**
	Min-max	**median**	**median**	**median**	**median**
Tropical (0°-25°)					
Lakes and reservoirs	1840-1840	1900	4.0	240	0.45
Rivers (> 60-100 m wide)	146-146	3600	12.3	1600	0.23
Streams (< 60-100 m wide)	60-60	4300	17.2	2720	0.16
Wetlands	3080-6170	2900	2.4	240	1.12
Temperate (25°-50°)					
Lakes and reservoirs	880-1050	900	4.0	80	0.08
Rivers (> 60-100 m wide)	70-84	3200	6.0	720	0.05
Streams (< 60-100 m wide)	29-34	3500	13.1	2630	0.08
Wetlands	880-3530	2500	2.4	210	0.47
Boreal and Arctic (50°-90°)					
Lakes and reservoirs	80-1650	110	4.0	130	0.11
Rivers (> 60-100 m wide)	7-131	1300	6.0	260	0.02
Streams (< 60-100 m wide)	3-54	1300	13.1	560	0.02
Wetlands	280-5520	200	2.4	170	0.49
Global	**Area of inland waters (1000s km^2)**	**Percent of global land area**		**Zonal outgassing (Pg C yr^{-1})**	
Lakes and reservoirs	2800-4540	2.1-3.4		0.64	
Rivers (> 60-100 m wide)	220-360	0.2-0.3		0.30	
Streams (< 60-100 m wide)	90-150	0.1-0.1		0.26	
Wetlands	4240-15,220	3.2-11.4		2.08	
All inland waters	**7350 - 20,260**	**5.5 - 15.2**		**3.28**	

Latest estimates on carbon emissions from inland waters, highlight a very large contribution to overall CH_4 and CO_2 emissions from very small ponds. Of the 0.571 Pg C yr^{-1} of CO_2 and 0.012 Pg C yr^{-1} of CH_4 released, it is estimated that small ponds contribute 15.1% and 40.6% to total CO_2 and CH_4 emissions respectively (Holgerson & Raymond, 2016). Both CO_2 and CH_4 concentrations in smaller inland waters have displayed super saturation (Natchimuthu et al., 2014; Kankaala et al., 2013; Laurion et al., 2010). High loadings of terrestrial dissolved organic carbon (DOC) and OC in relation to overall water volume, lead to high respiration rates and CO_2 production (Holgerson & Raymond, 2016; Kortelainen et al., 2006). CH_4 concentrations also appear greater in small water bodies (see figure I.1). High productivity and sediment anoxia, coupled with reduced CH_4 oxidation rates in the water column due to shallow depth, lends itself to high efflux of CH_4 (Holgerson & Raymond, 2016; Bastviken et al., 2004).

Furthermore, the temporary nature of many water bodies at this size range leads to complex patterns in microbial activity and remineralisation. Oxygen exposure in dry periods is known to favour rapid mineralisation of OC from these systems (Sobek et al., 2009), transitional periods of recharge and inundation can also result in intense release of CO_2 (Gilbert et al., 2016; Catalán et al., 2014). It has been suggested that CO_2 and CH_4 emissions from water bodies <0.01km^2 may be equal to that of larger lakes at localised and global scales (Abnizova et al., 2012; Torgerson and Branco, 2008). To understand the potential role of small water bodies in GHG emissions, we first need to explore the key factors driving the release of these gases.

6.2.2 Degradation

Degradation of OM is largely dependent on two key factors: i) Chemical composition and molecular structure; ii) Environmental conditions upon deposition. OM that is molecularly heavier in composition is more refractory than smaller chained molecules that are readily degraded by microbial communities or undergo photo-oxidation quite quickly (Aichner et al., 2010). Allochthonous OM may contain more lignin and cellulose based molecules, which are notably resistant to degradation. Whereas autochthonous OM, particularly algal based, can often undergo degradation and remobilisation by microbial communities in the water column, before deposition within sediments (Attermeyer et al., 2014; Downing, 2010). Observed patterns in preferential degradation of autochthonous OM (Ferland et al., 2014) and formation of anoxic sediments due to high productivity, indicate that the proportion of allocthononous and autochthonous OM inputs has a substantial affect on rates of burial and subsequent remineralisation (Reverey et al., 2016).

Environmental conditions play an important role in the degradation of OC and are a major determining factor in whether OC is released back to the atmosphere as either CO_2 or CH_4. In oxic conditions, OM is broken down and obligate aerobic heterotrophic microbial communities, in either the water column or the upper sedimentary layers, produce CO_2. These microbial communities are limited to oxic or "active" layers within the sediment and are therefore influenced by sediment disturbance and bioturbation.

In eutrophic environments, the water column and sediment layers quickly become depleted in oxygen due to increased OM loadings to the sediment. Anoxic conditions promote activity from methanogenic microbial communities. Anaerobic communities operate at a much slower metabolic rate, with rates of CH_4 release much lower than those of CO_2. However, CH_4 is a more potent GHG with a warming effect 25 times greater than CO_2 (Durocher et al., 2014). A study carried out on wetland environments, the largest natural atmospheric source of CH_4, suggests that anaerobic CH_4 oxidation potentially reduces CH_4 emissions by around 50% per year (Segarra et al., 2015). Other studies on carbon dynamics in wetland environments, have suggested that after around 300 years, CH_4 emission is irrelevant in comparison to OC sequestration, as they often become both a net source and sink (Mitsch et al., 2014; Mitsch et al., 2013).

6.2.3 Microbial activity

Freshwater ecosystems including wetlands and ponds are eminently microbial based. Ecosystem functioning is heavily reliant on communities of bacteria, archaea, fungi and protists (Peralta et al., 2013; Reddy and DeLaune, 2008; Briée et al., 2007; Hahn, 2006). The biogeochemical importance of microbial communities was first appreciated in the 1940s. Lindeman (1942) acknowledged "microbial ooze" as a central component of the trophic dynamics in Cedar Creek Bog (Lindeman, 1942). From this early recognition, it is now well understood that bacterial activity drives biogeochemical transformations and cycling of biologically active elements, such as carbon, nitrogen and sulphur, within inland waters (Newton et al., 2011; Briée et al., 2007).

The role of microbes as primary degraders and mineralisers of organic compounds to their inorganic constituents is of renewed interest now the more substantial role of inland waters in the global carbon cycle has been recognised (Newton et al., 2011). Research has highlighted susceptibility to changing trophic states and altered ecosystem functioning (Reverey et al., 2016; Heathcote and Downing, 2012; Aufdenkampe, 2011; Downing, 2010). Understanding microbial community structure and diversity is central to determining relationships between environmental conditions and ecosystem functioning (Sims et al., 2013; Peralta et al., 2013).

OC storage within soils represents the balance between plant and root litter production, root exudates and subsequent decomposition (Lange et al., 2015). Enhanced OC storage can be attributed to enhanced primary production (Lange et al., 2015; Marquard et al., 2009; Tilman et al., 2001) or longer residence time of OM due to slower rates of degradation (Lange et al., 2015; Jastrow et al., 2007). Microbial activity can influence this via a number of mechanisms. Large amounts of labile OM deposition can accelerate decomposition through the "rhizospheric priming affect". Increased inputs of labile carbon, results in a more active and abundant microbial community (Lange et al., 2015; Kuzyakov, 2002), ultimately amplifying OC degradation (Shackle et al 2000). Alternatively or perhaps in parallel, increased microbial activity and necromass accumulation can promote enhanced OC storage (Lange et al., 2015).

Given the degree of specialisation of bacterial groups within the carbon cycle (Strickland et al., 2009), understanding environmental factors and associated bacterial communities across ecosystems, such as ponds, should reveal linkages between physical and trophic characteristics, and improve our understanding of observed variability in GHG emissions. (Neghandi et al., 2014). Large uncertainties exist on the communal role of aquatic microbes in GHG cycling, yet it is becoming increasingly well documented that microbial community composition plays a significant role in determining ecosystem process rates (Neghandi et al., 2014; Strickland et al., 2009; Reed and Martiny, 2007). However, little is known about microbial communities present within small water bodies, especially considering the substantial heterogeneity observed across these habitats and research documenting the intensity of biogeochemical processes occurring within them (Hahn, 2006).

6.2.4 Vegetation and carbon fluxes

The presence of vascular plants is recognised as a key factor determining rates of CO_2 and CH_4 fluxes. They affect a number of processes related to transport, production and consumption of CO_2 and CH_4 within inland waters. The heterogeneity of vegetation communities observed between aquatic systems, particularly ponds, alongside spatial variations in the distribution of plant communities within individual ponds, leads to a wealth of implications when attempting to upscale flux estimates across regional scales (Strohm et al., 2007; Joabsson et al., 1999). Studies aiming to understand the role of vegetation in gas flux dynamics, observed distinct differences in the functioning of various species in relation to both CO_2 and CH_4 fluxes. Different plant species demonstrated different rates of CH_4 ebullition, rhizospheric oxidation of CH_4 to CO_2 and stimulation of methanogenesis through root exudates (Strohm et al., 2007).

Vegetation succession in ponds further compounds our ability to upscale carbon emission estmates. In any given coverage of ponds, information on age, and past vegetation succession will likely be limited, despite these factors having a substantial influence on carbon fluxes. Development of vascular plant based communities can lead to oxidation of rooted sediment layers, minimising anaerobic conditions and the production of CH_4 (Couwenberg, 2009). Studies on peatlands have reported that mosses, such as *Sphagnum* restrict emissions, whilst *Juncus* promote enhanced carbon emissions due to the labile composition of root exudates, resulting in enhanced microbial activity (Lange et al., 2015).

Such complex dynamics as a result of extensive vegetation coverage observed in small water bodies, has led some to believe that they are more terrestrial in terms of their behavioural functionality (Cole et al., 2007), and may account for their absence in carbon budgets for inland waters.

6.2.5 Hydro-period and carbon fluxes

Patterns of drying and rewetting lead to complex biogeochemical cycling of nitrogen, phosphorus and carbon in small water bodies (Reverey et al., 2016). Emerging research has highlighted rapid remineralisation of CO_2, from temporary aquatic systems under hydrological regime change, particularly the transition from inundation to dry (Gilbert et al., 2016; Catalán et al., 2014) and its effect on the redox dynamics of sediments (Reverey et al., 2016). The transition from dry to wet periods has also been shown to impact the remobilisation of NO_3^- and PO_4^{3-} (Reverey et al., 2016).

Dry-wet cycles lead to rapid changes in the redox regime due to increased aeration of previously inundated sediments (Reverey et al., 2016). Aeration increases the redox potential of sediments and pore water, enhancing microbial activity and carbon remineralisation. Photodegradation and exposure to UV radiation also alter the chemical composition of OM, degrading recalcitrant fractions to produce CO_2 and more labile OM, enhancing microbial activity and carbon remineralisation (Reverey et al., 2016; Lange et al., 2015; Aichner et al., 2010). However, during longer periods of desiccation, microbial activity often becomes limited due to decreasing moisture availability, which can kill up to 70% of microbial biomass, whilst also restricting activity due to energy redirection to strategic mechanisms to deal with drought stress (Reverey et al., 2016; Schimel et al., 2007). Longer drought periods can also impair microbial activity upon pond rewetting as communities recover, which limits remineralisation of carbon.

Although there is contradictory discussion, there is a general consensus that microbial communities adapt to drought stress. Species with higher tolerance to disturbance, such as facultative microbes (Baldwin & Mitchell, 2000), or microbes able to adapt, such as Actinobacteria, which are resilient to drought stress through spore formation (Cupples, 2013), will be favoured (Reverey et al., 2016). Communities accustomed to frequent dry-wet cycles can recover more quickly upon rewetting. However, prolonged desiccation has been shown to shift freshwater bacterial communities towards that of a soil (Pohlon et al., 2013). The hydrological history, i.e. the duration and frequency of previous dry-wet cycles, alongside associated redox dynamics, therefore plays an important role in the magnitude of microbial and biogeochemical responses to changing hydrological conditions (Reverey et al., 2016; Peralta et al., 2013).

There are clear links between hydro-period, microbial community structure and biogeochemical processing. As previously discussed hydro-period is largely driven by local climate, pond type and surrounding land use. The future ecosystem functionality of small ponds is therefore likely to be highly dependent on climatic behaviour, changing in susceptibility depending on pond type and location. A wetter climate could lead to increased numbers of small water bodies, increasing carbon cycling and promoting preservation of OC by limiting oxygen exposure time. However, a warmer, drier climate could lead to the loss of temporary water bodies from our landscapes and an increase in the number of permanent features undergoing dry-phases, resulting in desiccation of previously inundated sediments and remineralisation of current carbon stocks. Further research into the hydrological and biogeochemical response of ponds, to current and projected future climate change, is critical to understanding their role in the carbon cycle and in identifying the potential to engineer constructed ponds, for effective, strategic carbon mitigation, functionaliy resilient to future climate change.

7.0 Summary

Ponds are intrinsic and ubiquitous components of our landscapes. While there is no standardised definition as to what actually constitutes a pond and the boundaries between small lakes, wetlands and ponds are often blurred. Residence times can be over geological timescales such as the postglacial Pingo and Kettle Hole ponds, or be a few decades, such as ponds created by land subsidence. Previously overlooked, their ecological importance has now been realised after a surge in research on pond ecology. However, research on the biogeochemical functioning of ponds is limited due to a large bias towards the study of larger lakes and reservoirs. Technological advancements have improved the resolution at which we can map such features and it is now believed they occupy a much greater global coverage than previously thought, at around 3.7% of the earth's non-glaciated land surface. Alongside growing evidence demonstrating disproportionately intense rates of biogeochemical cycling, it is becoming increasingly apparent they are a substantial component within the global carbon cycle and could potentially be utilised as natural carbon mitigation features.

> *"Society will need to continue further, to negative emissions. That is removing carbon dioxide from the atmosphere and storing it somewhere else. There are various options here, from planting trees and keeping restored forest in perpetuity, enhancing uptake in soils..."*
>
> *COP 21 2015*

Our current understanding of the role in which small ponds play in carbon cycling and storage is restricted by limited research into this area. Few studies have published quantified results on the amount of OC currently stored within these systems, let alone rates of OC burial and fluxes to the atmosphere. If ponds are to be realised as a substantial component in global carbon budgets and included in environmental policy mechanisms, such as land sparing and CAP EFAs, it is crucial to accurately quantify rates of biogeochemical processing and elucidate dominant driving factors. The following chapters will focus on these key components. Only then can they be integrated into comprehensive global carbon budgets and engineered at the landscape scale to provide targeted ecosystem functioning.

8.0 Project Overview

A brief overview of the following chapters is outlined below:

Chapter II	An overview of the experimental study site and the historical development of ponds sampled as part of this study.
Chapter III	An overview of key methods and calculations used over the following chapters.
Chapter IV	A novel approach to evaluate the accuracy of using sediment cores to estimate whole system OC storage and burial rates. OC storage and burial within the ponds in comparison to the surrounding soil has also been assessed.
Chapter V	A review of the physicochemical development and dynamics across 3 newly constructed ponds over a 3 year period. This chapter focuses on elucidating dominant factors driving productivity and exploring OC storage and burial over this initial stage of succession.
Chapter VI	An assessment of OC storage and burial within 9 ponds across the study site belonging to 3 distinct vegetation groups. The chapter seeks to provide an accurate value for OC burial within the small ponds and identify its relationship to past vegetation community succession.
Chapter VII	An exploration into microbial diversity and community structure between different ponds and down the core profile. This chapter seeks to provide novel insights into bacterial communities present within the ponds, whilst exploring the impact of physicochemistry, vegetation coverage and implications for carbon storage.
Chapter VIII	Final reflections on key results outlined in the study and a contextualsation of results with respect to the global carbon cycle and landscape carbon mitigation measures.

Chapter II – Study Site: Hauxley Nature Reserve, Northumberland, UK

1.0 Druridge Bay

The study site for this research lies within the Druridge Bay region of Northumberland, UK (see figure II.1). Northumberland largely comprises of upland and moorland environments, situated within and around the Cheviot Hills. However, adjacent to the coast lays a stretch of sand dunes and productive lowland, dominated by agricultural activity. The coastal plain in which Druridge Bay is located, has a cool, dry, temperate climate, with maximum mean summer temperatures rarely exceeding 20°C. Rain shadow from the hills to the West results in fairly moderate rainfall in comparison to other areas of the county, usually <800mm yr^{-1} (Gilbert et al., 2014; Lunn, 2004), although this is largely subject to the influence of the North Atlantic Oscillation (George et al., 2004; Fowler and Kilsby, 2002).

Figure II.1: Druridge Bay study site location.

Druridge Bay lies within the area between Amble in the north (Lat: 55.336468, Long-1.5790701) to Cresswell in the south (Lat: 55.235092, Long: -1.5395880). Although largely utilised for agriculture purposes in the present day, the lowland coastal plain has a strong mining heritage. Upon closure, several large opencast mines in the area were converted into nature reserves and country parks under local biodiversity conservation strategies. This has created a complex of rich wetland habitats along the bay that occupy roughly 10% of the area (Jeffries, 2012). Subsidence from underlying

coal mine-shafts has resulted in a number of depressions forming across the region, leading to the development of features, such as Druridge Pools near Cresswell, that augment wetland habitats created upon the remediation of the coal mines. Smaller ponds and dune slacks >400m^2, currently occupy approximately 2% of the landscape area. Distinct seasonal extremes within the region of prolonged rainfall and warmer, drier summer months, create a mosaic of small pools and flashes <10m^2, that come and go annually. The temporal behaviour of these small wetlands results in large inter-annual variations in the number and coverage of water bodies (Jeffries, 2016; 2012).

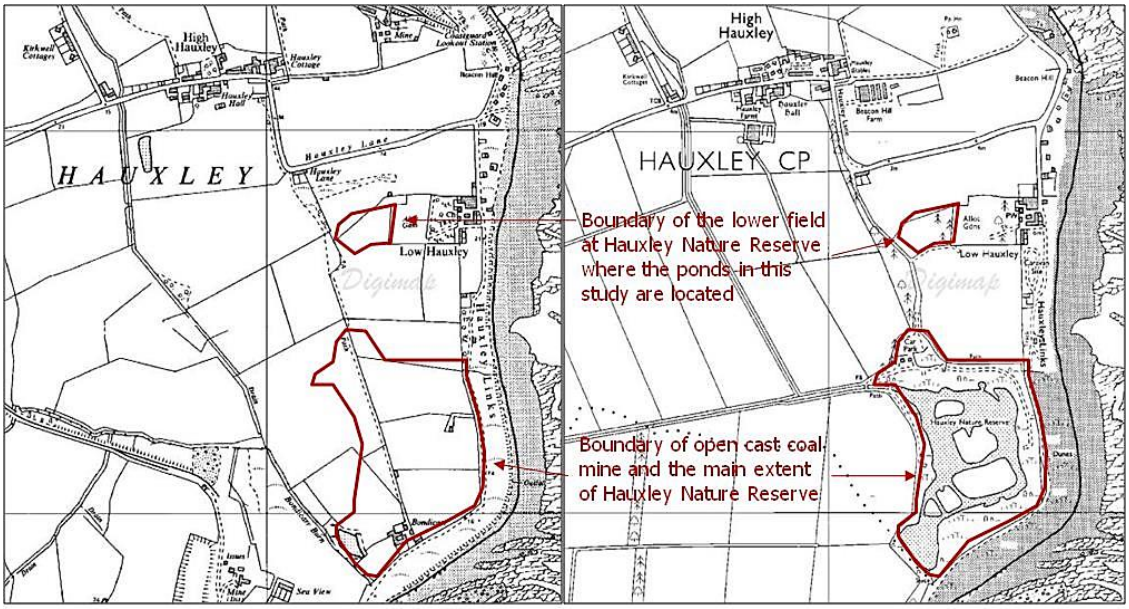
The conservational importance of ponds in Northumberland is gaining increasing recognition at both National and International scales. At regional scale ponds, wetlands, and reed beds are seen as increasingly important features for a number of amphibians as well as coastal, farmland and upland bird species, in the Northumberland Biodiversity Action Plan (Jaggs, 2008). The large number of ponds across Druridge Bay coupled with the large degree of heterogeneity observed in macrophyte and macroinvertebrate communities between ponds (Jeffries, 2015, 2010), highlights the value of this area in the maintenance of Northumberland's biodiversity. Ponds in the area are subject to increasing agricultural pressure. Seasonal flooding of arable cropland can have substantial financial implications for local farmers, leading to infilling and drainage of depressions, highlighted as a significant cause of the loss of wetland environments in the Northumberland Biodiversity Action Plan (Jaggs, 2008). Demonstration of the environmental and potentially financial value of other ecosystem services offered by wetland environments, such as flood mitigation and carbon storage, may offer farmers incentives to conserve and restore these valuable habitats.

2.0 Hauxley Nature Reserve

Hauxley Nature Reserve is located at the northern end of Druridge Bay, Northumberland UK (British National Grid NU 285 025, 60°27'61"N; 42°85'48"E) see figure II.1. Hauxley Nature Reserve comprises of a central reserve area (figure II.2(2)), a lower field (figure II.2 (3))and one main pool, with islands and reed beds (figure II.2(1), to name just a couple of features and habitats that have been created upon remediation of the open cast coal mine (see figure II.2). Figure II.3 shows the study site in 1960 as Radcliffe opencast coalmine and Hauxley Nature Reserve as it is now. A number of ponds and smaller wetland areas are commonplace throughout the reserve, which support a wealth of insect, bird and plant species. The study site for this research comprises of a field located at the lower end of the reserve.

Figure II.2: Aerial Image displaying 1) main reserve pool 2) central reserve area and visitors centre 3) lower field experimental pond site.

Figure II.3: Historic Land use maps showing the opencast coalmine site in 1960 and the creation of the nature reserve in the 1990s. Maps taken from Edina Digimap.

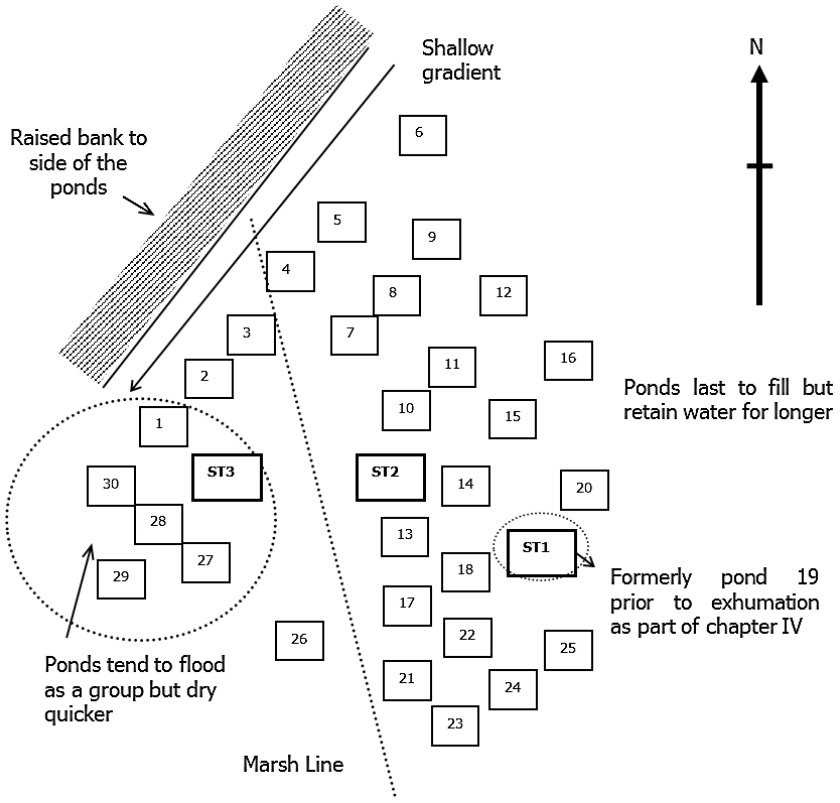


2.1 Constructed Ponds

In the autumn of 1994, thirty small ponds were constructed in a roughly triangular array across the field's gradient of seasonal inundation, roughly 10 – 50m of larger more permanent ponds (Jeffries, 2008). See figure II.4 and II.5 for a detailed site map and conceptual site plan).

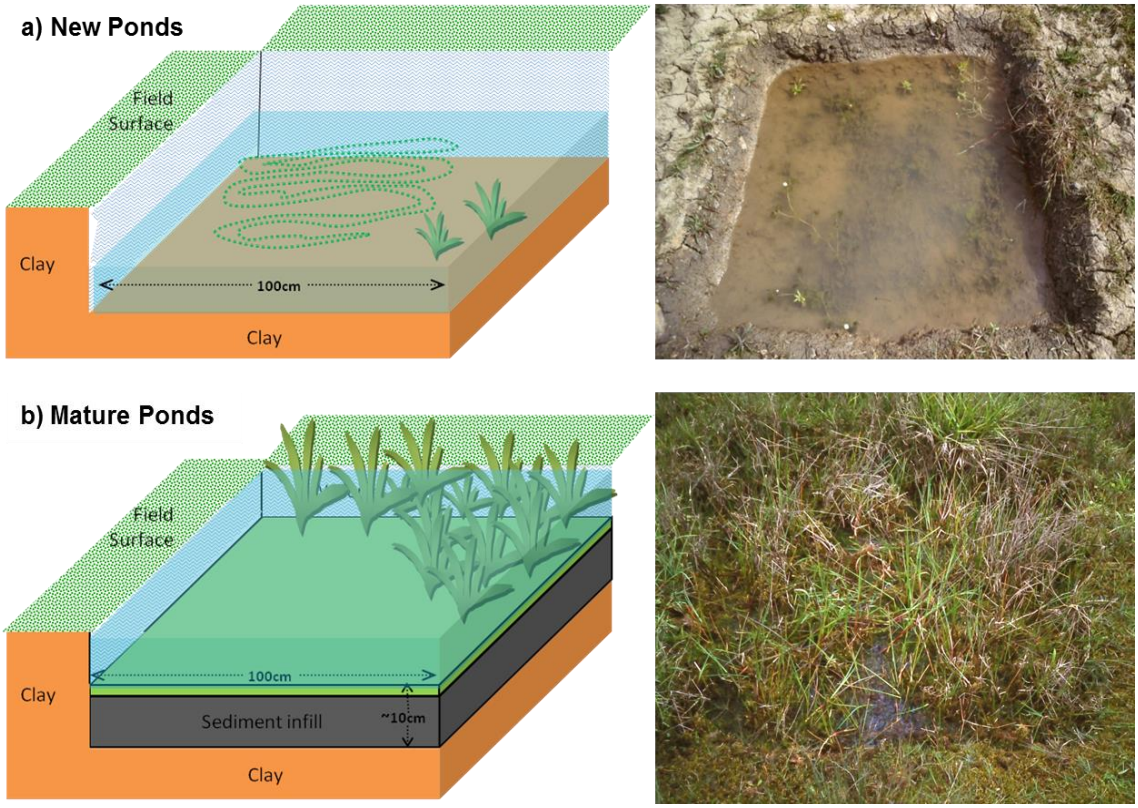
Figure II.4: Aerial image showing the experimental pond array and the two larger permanent ponds at the Hauxley field site.

Figure II.5: Conceptual Site Plan.



Each pond was constructed to be around 1m^2 and around 30 – 40cm deep, over an area of around 30m x 30m, in an attempt to create as close to replicate ponds as is possible under natural conditions. The ponds are temporary and are often subject to dry-phases throughout summer months, prior to inundation in autumn. (See figure II.6 for images of the ponds and conceptual pond model).

Figure II.6: Conceptual model of a) newly constructed/ early succession pond and b) Mature late succession pond.



Ponds were constructed in an area of the field vegetated with thin, patchy swards of *Leptodicytum riparium*, *Agrostis stolinifera*, *Alopecurus geniculatus* amongst extensive bare substrate. Adjacent to the ponds, were areas of *Eleocharis palustris* and *Agrostis Stolinifera,* which, in wetter years, would be classified under the UK National Vegetation Classification as S19 *Eleocharis* swamp (Jeffries 2008; Rodwell, 1995). Following particularly wet summers in 1997-1998, the area of *E.palustris* and *A.stolonifera* expanded into a distinct, closed sward around ponds at the southern edge of the array, whilst ponds at the northern edge remained in amongst more terrestrial vegetation (see figure II.7). This formed a distinctive marsh line across the site separating ponds (1, 2, 3, 26, 27, 28, 29, and 30) from those at the higher end of the site. The development of the marsh line can be seen in figure II.8, which highlights

more generalised vegetation across the site in more recent years as the climate has become drier.

Figure II.7: The Hauxley pondscape: spatial array of the 30 ponds. The blue area represents the initial extent of *Agrostis stolonifera* and *Eleocharis palustris* sward, and the red area represents extent of the sward after wet years 1997 and 1998, upon the formation of the marsh line. The arrow shows the direction from which winter inundation spreads out north-eastward over the array of ponds from start point amongst south-western ponds. Adapted from Jeffries (2008).

Figure II.8: Photographs of the site showing the development of the distinct marshline.

The ponds were constructed originally to match ponds created from the removal of anti-tank blocks, which displayed high β diversity (Jeffries, 1994). However, the history and community variation of these ponds was unknown. Constructing new ponds at Hauxley Nature Reserve allowed the role of historic events to be monitored, in order to assess whether β diversity was a result of stochastic or more deterministic processes. The Hauxley ponds have since formed the basis of numerous studies into the development and spatial heterogeneity of macrophyte and macro-invertebrate communities, particularly in response to local climate and patterns of wetting and drying (Jeffries, 2010; 2008; 2005; 2003; 2002).

Exploring the effect of vegetation community succession on OC burial is a key concept, as ultimately this determines the proportions and type of OM available for storage. Previous studies have reported results for the first ten years of the ponds existence (Jeffries, 2008). Results have demonstrated that ponds display substantial spatial and temporal heterogeneity in the development of macrophyte communities, which may have a potentially significant effect on OC burial. However, to determine this we first need to look at vegetation community succession throughout the entire ponds history

3.0 Vegetation Community Succession

Pond vegetation was recorded annually in late May/early June by Dr Michael Jeffries, since the initial construction of the ponds in 1994. Sampling was carried out using a $1m^2$ point quadrat with 81 point samples per pond. Vegetation beneath each grid-wire intersection was recorded as species apart from *Agrostis stolonifera* and *Alopecurus geniculatus,* which could not be separated with reliability and so have been treated as one taxon, and will hereby be referred to as *Agrostis*. Small terrestrial herbaceous seedlings too small to identify have been recorded as “unidentified seedlings”. Results up until 2004 have been previously published (Jeffries, 2008). The results presented in this chapter have were produced using the updated dataset covering 1995-2014.

Twenty-two species of macrophytes and eight herbaceous terrestrial species were recorded in the ponds from 1995 to 2014 (See table II.1 for full list of recorded species). However, only nine species were widespread across the site or formed extensive communities within ponds; *Spirogyra* sp., *Chara vulgaris*, *Leptodicytum riparium*, *Ranunculus aquatilis*, *Juncus articulatus*, *Glyceria fluitans*, *Agrostis stolonifera* and *Eleocharis palustris*. Six of the twenty two species were herbaceous terrestrial plants that colonised ponds throughout dry periods as relatively small seedlings e.g. *Ranunculus repens.*

Table II.1: List of species observed across the site. Species highlighted in bold displayed the most substantial coverage across the ponds.

Species	Common Name
Agrostis stolonifera	**Creeping Bentgrass**
Alopecurus geniculatus	**Marsh/Water Foxtail**
Alisma plantago aquatica	Common Water Plantain
Callitriche sp	Water Starwort
Cardamine sp	Bittercress
Carex Glauca	**Glaucous Sedge**
Carex ortubae	False Fox Sedge
Chara vulgaris	**Common Stonewort**
Cirsium sp	Thistles
Eleocharis palustris	**Common Spike-rush**
Epilobium palustris	Marsh Willow Herb
Equisetum arvense	Common horsetail
Galium palustris	Common Marsh Bedstraw
Glyceria fluitans	**Floating Sweetgrass/ Water Mannagrass**
Juncus articulatus	**Common jointleaf rush**
Juncus inflexus	Hard Rush
Leontodon sp	Dandelion
Leptodicytum riparium	**Kneiff's Feathermoss**
Mentha aquatica	Water Mint
Mysostis sp	Forget-me-nots
Plantago major	Broadleaf/ Greater Plantain
Potamogeton berchtoldii	Small Pondweed
Potentilla anserina	Common Silverweed
Pulicaria dysentaria	Common Fleabane
Ranunculus aquatilis	**Common Water Crowfoot**
Ranunculus repens	Creeping Buttercup
Rumex crispus	Curled Dock
Schoenoplectus lacustris	Club Rush
***Spirogyra* sp**	**Filamentous Algae**
Trifolium repens	White Clover

TWINSPAN analysis was performed to separate ponds into groups. Four distinct groups were identified after two rounds of analysis, corresponding to changes in dominant and sub dominant vegetation communities (Table II.2). The first division separated ponds dominated by swards of *L.riparium* (Group A) and those dominated by *C.vulgaris* or *Spirogyra sp* (Group B). Subsequent divisions separated ponds into one of four groups based on sub dominant communities (e.g. Group 1 are ponds dominated by *L.riparium* with coverage of *G.fluitans* or *R.crispus*, whereas group 2 were also dominated by *L.riparium* but displayed sub dominant communities of *A.stolonifera*, *J.articulatus* and *C.glauca*. Group 3 and 4 are ponds dominated by *C.vulgaris* or *Spirogyra*. Group 3 displayed extensive coverage of *R.aquatilis* and *Callitriche*, as opposed to coverage of *E.palustris* and *J.articulatus* in Group 4.

Table II.2: TWINSPAN defined groups for vegetation community succession.

Group A **Primary Indicator species:** *Leptodicytum* sp. (Moss) **Secondary Indicator Species:** *Agrostis/Alopecurus* *Carex glauca* *Eleocharis palustris* *Glyceria fluitans* *Rumex crispus*	**Group 1** *Glyceria fluitans* *Rumex crispus* 
	Group 2 *Agrostis/Alopecurus* *Carex glauca* *Juncus articulatus* 
Group B **Primary Indicator Species:** *Chara vulgaris* *Spirogyra* sp. (Filamentous algae) **Secondary Indicator Species:** *Callitriche* *Ranuculus aquatilis*	**Group 3** *Callitriche* *Ranuculus aquatilis* 
	Group 4 *Eleocharis palustris* *Juncus articulatus* *Ranuculus aquatilis* 
Pond Exhumed	

The Hauxley ponds displayed generally similar patterns of vegetation community succession. However, individual plant species and communities showed extensive spatial and temporal variation over the twenty years. See figure II.9 for distributions of TWINSPAN groups between ponds in 1995, 1998, 2001, 2004, 2006, 2008, 2011 and 2014 (see appendix figure IX.1 For full 1995 – 2014 TWINSPAN group plots). Examples of species incidence can be seen figure II.10 and percentage cover in figure II.11.

Figure II.9: Twinspan plots showing vegetation development across the site at roughly 3 year intervals. Symbols represent grous define in table II.2.

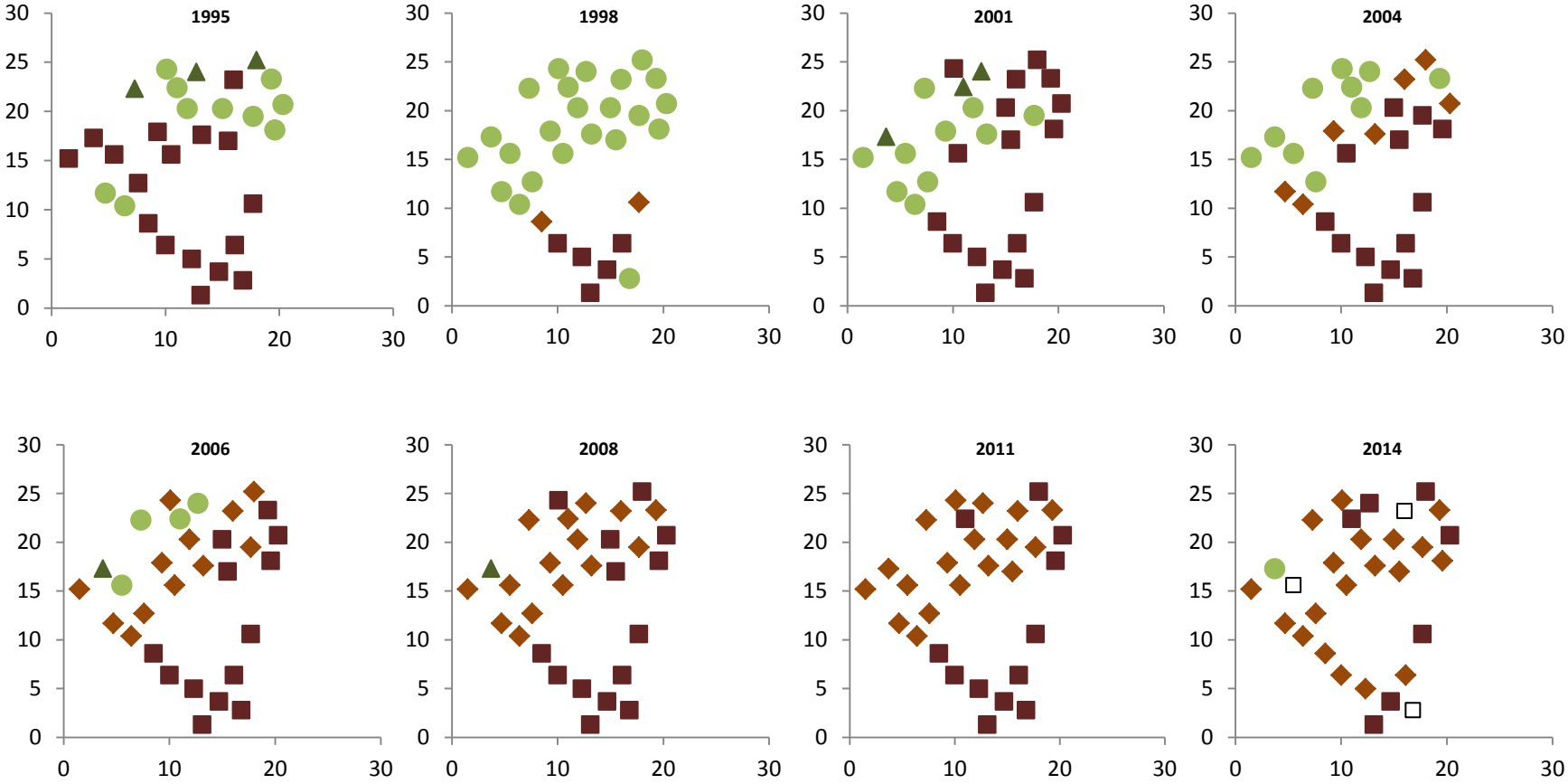


Figure II.10: Incidence of plant species in the thirty ponds 1995-2014.

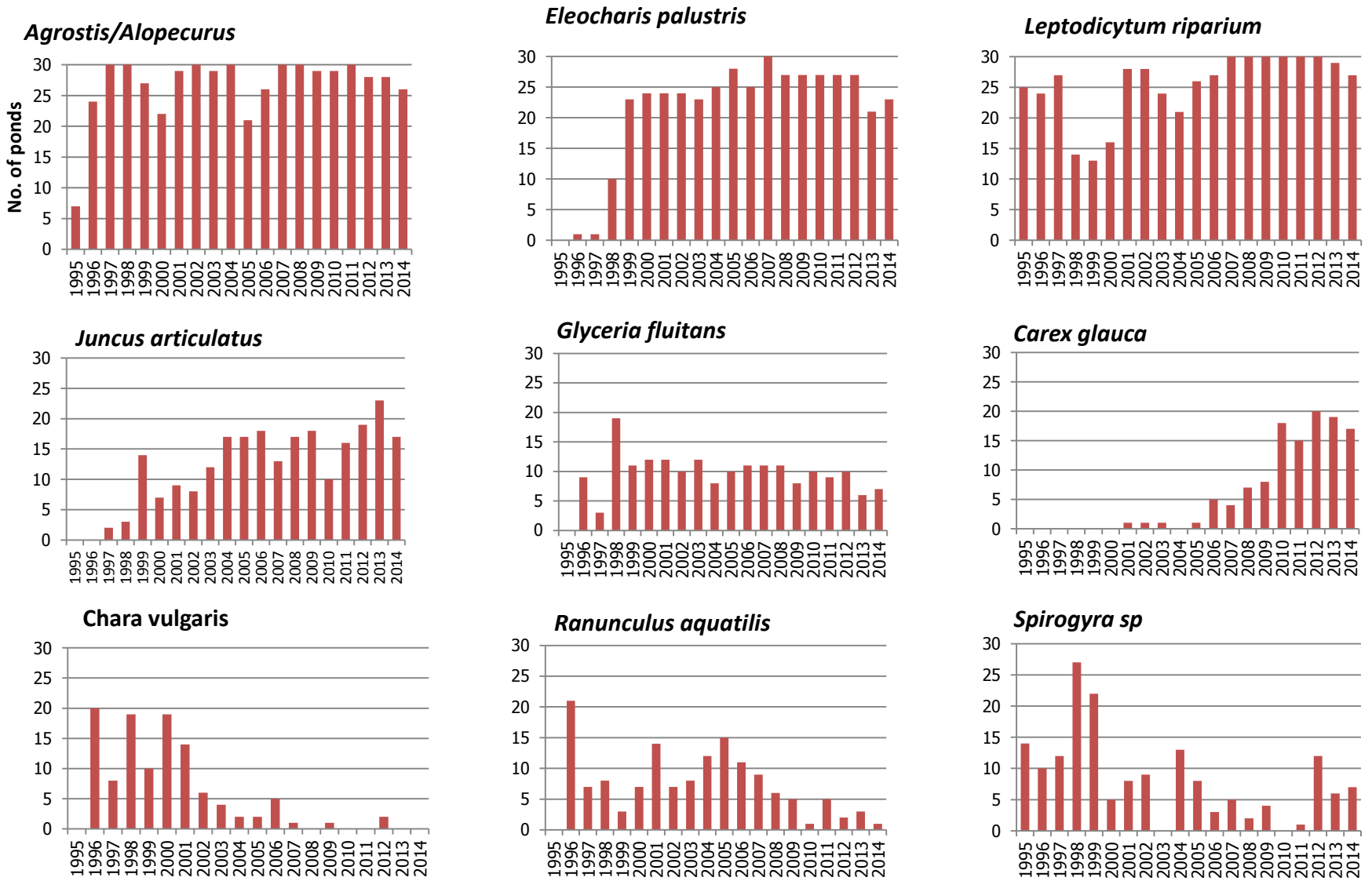
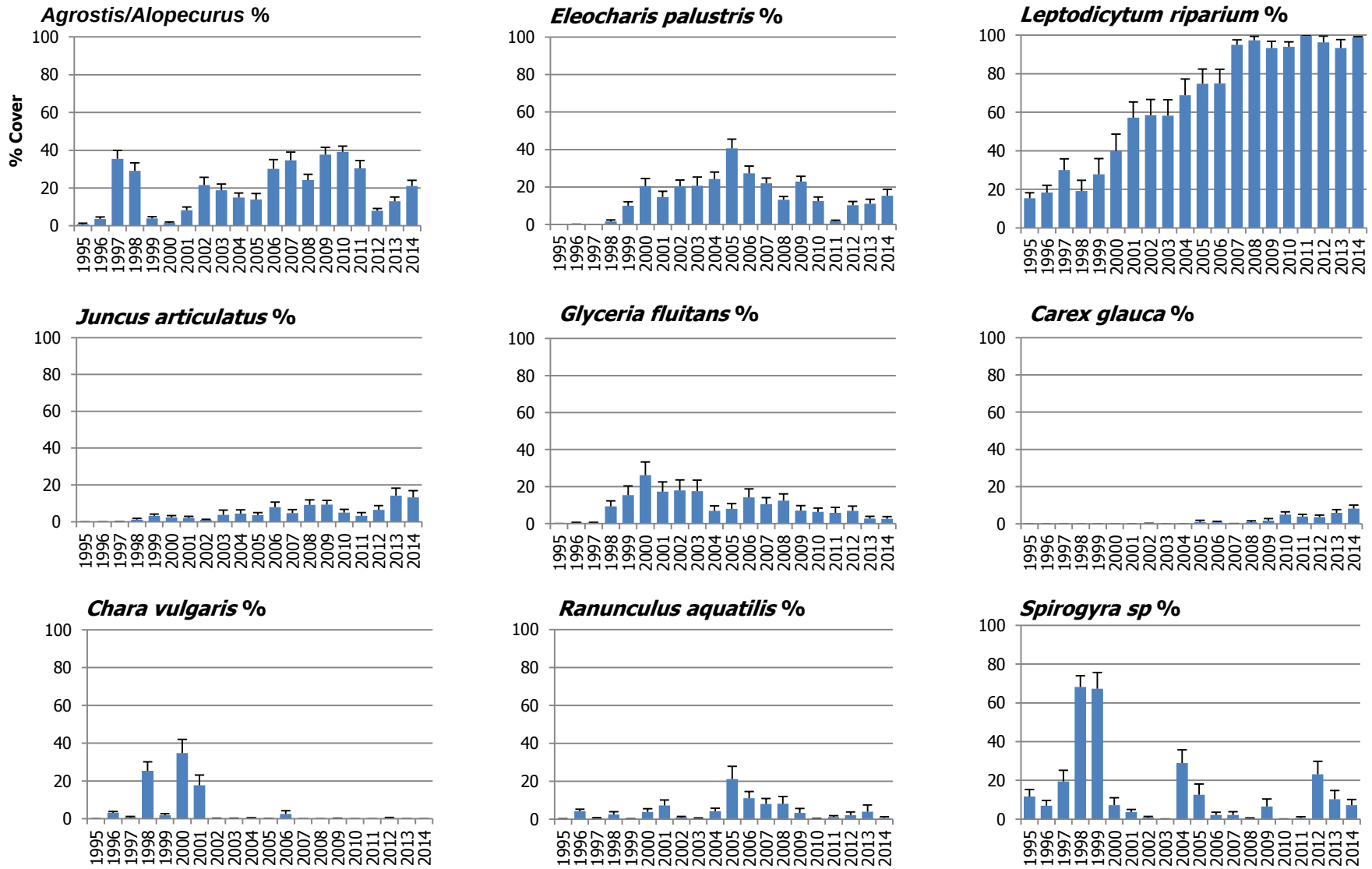


Figure II.11: % Cover of plant species in the thirty ponds 1995-2014.



In the first year, the majority of ponds retained bare bottom substrates with scattered strands of *L.riparium* or strands of *Agrostis* encroaching from the surrounding area, although some species were widespread across the ponds (e.g. *Spirogyra* and *L. riparium*, figure II.9 and II.10). By 1996 aquatic based communities of *C.vulgaris*, *Callitriche* and *R.aquatilis,* which are often described as pioneer species (e.g. Fleury and Perrin, 2004), had established and were widespread across the site, alongside *L.riparium* and *A.stolonifera*, although % cover remained patchy (figure II.11).

Temporal patterns were also observed, such as the wet summers of 1997-1998. The lack of dry-phase promoted the development of thick blankets of *Spirogyra*, which limited the development of other species, particularly the spread of *L.riparium* (see figure II.10). Throughout these wet years the spread of moss dominated communities appeared to halt, due to extensive growth of *Spirogyra*. Upon return of seasonal dry-phases in 1999, extensive *Spirogyra* mats did not return until wetter years and plant communities that had been affected began to recover. This was most apparent in the continued spread of *L.riparium* north easterly across the site (figure II.9).

Distinct communities became apparent from around 1998 onward. Substantial differences were observed between ponds with substrate covered by *L.riparium*, often with sub dominant communities of *G.fluitans* or *E.palustris*, and ponds whose substrate remained fairly exposed, which supported communities of *Chara vulgaris* and *Ranunculus aquatilis* (figure II.9). Initially this divide was most apparent between either sides of the marsh line (see figure II.9). Ponds on the south western side of the array, in amongst the *Agrostis* and *E.palustris* area, were dominated by extensive swards of *L.riparium*, whilst those on the northeastern side supported aquatic communities of *Spirogyra*, *C.vulgaris* and *R.aquatilis*.

Moss dominated communities spread north easterly across the array of ponds and by 2004, had developed extensive coverage in the majority of ponds at the site. Over a similar period, the incidence of submerged pioneer species such as *C.vulgaris* declines quite rapidly (figures II.10, II.11) as mid-late successional species such as *E.palustris* and *J.articulatus* become more widespread (figure II.9, II.11). Ponds supporting communities of *C.vulgaris* and *R.aquatilis* were marginalised along the north eastern edge of the array (figure II.9). However, in the following four years (2004 - 2008) *L.riparium* established dominant communities in these ponds coinciding with the decreased incidence and coverage of *R.aquatilis* (figures II.10, II.11) and a general loss of *C.vulgaris* from the site, despite sporadic appearances in 2009 and 2012 (figure II.10). From this period onward, the majority of ponds across the site were dominated by thick swards of *L.riparium* (figures II.9, II.11).

From 2004 onwards, different sub-dominant communities within *L. riparium* dominated ponds began to establish and become widespread across ponds in the eastern array. This transition was characterised by increased coverage and establishment of *Agrostis*, *J. articulatus* and *C. glauca*, coinciding with a decrease in the coverage of *E.palustris* and *G.fluitans* despite these species remaining present in many of the ponds (figures II.9, II.11). By 2006, the majority of ponds on the eastern array had transitioned into these Group 2 communities, apart from the remaining Group 3 and Group 4 ponds on the north eastern margin, roughly equal in number to Group 1 communities that had previously dominated. By 2008, all but one of the remaining Group B ponds had transitioned into Group 2 communities that now dominated ponds across the eastern array.

In years following this, the majority of ponds transitioned into group 2 communities, apart from a small number of ponds at the southern edge of the site (figure II.9). This community composition, alongside physical observations on the ponds, would suggest that these ponds have entered the final stages of succession, having accumulated large masses of sediment and thick swards of vegetation. Although they still hold water for many months of the year, the ponds contain little areas of open water and in drier periods, these ponds can be hard to distinguish from the terrestrial landscape.

3.1 Deterministic Development of Local Scale Vegetation Community Heterogeneity

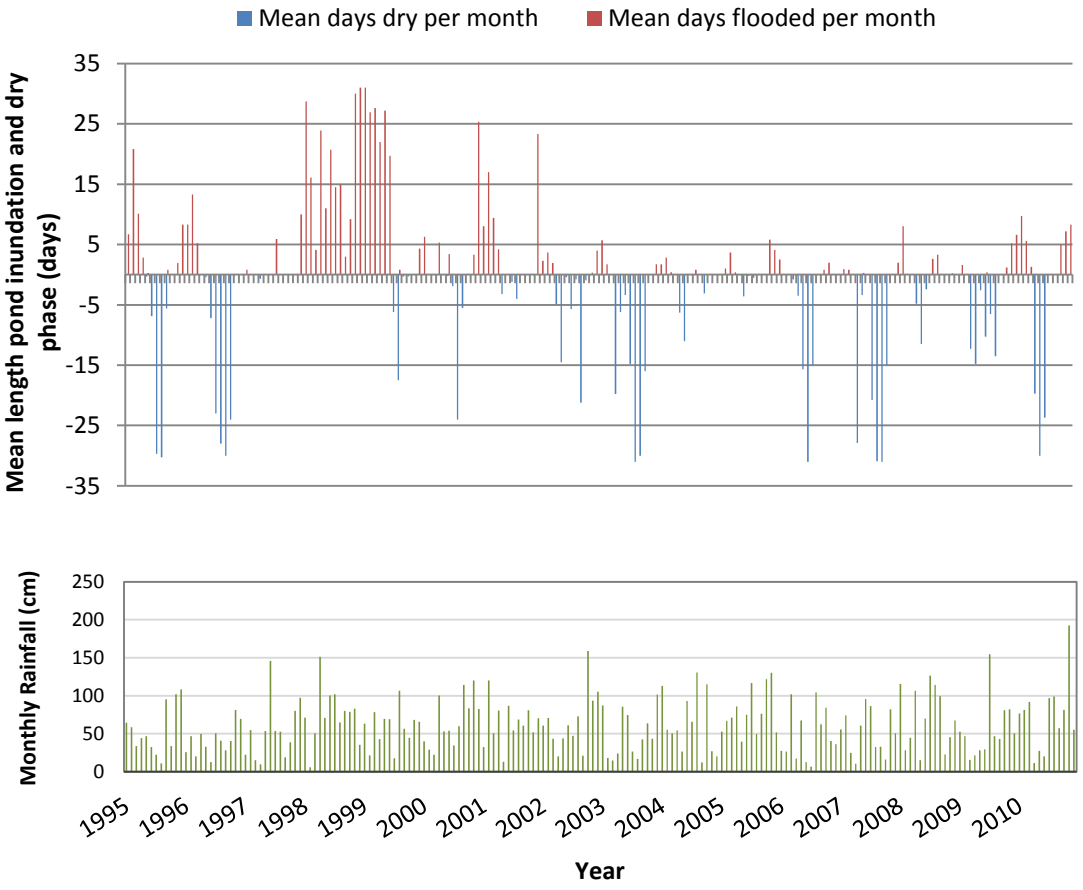
Spatial patterns of macrophyte community succession in the Hauxley ponds suggest an underlying deterministic factor, specifically the initial establishment of *L.riparium* dominated communities in south eastern ponds and subsequent changes as these communities spread across the site. Winter inundation is always longer in ponds at the south western area of the site coupled with the annual spread of floodwaters northeast over the ponds coincides with the spatial pattern of initial *L.riparium* dominant community establishment and its subsequent spread into other ponds. Following the wet years of 1997 and 1998 south western ponds were surrounded by a more distinct area of *A.stolonifera/E.palustris* wetland see figure II.8 around the same time *L. riparium* gained complete dominance in the enclosed ponds. As *L.riparium* was found in the majority of ponds from 1995 see figure II.9, it is unlikely that winter inundation facilitated the spread and establishment to other ponds. Spatial variations in inundation are principally a result of local geomorphology and spatial pattern of ponds at the site. Jeffries (2008) suggested that the *A.stolonifera/E.palustris* sward in the south western area and the spread of inundation outward from this zone, possibly created distinct physicochemical gradients due to deeper waters and more saturated substrate

facilitating the establishment of *L.riparium* dominated communities. This suggests that the spatial variation observed in plant communities is largely determined by the precise location of the pond within the field and how this determined an individual ponds hydrology, with small-scale differences between ponds creating distinct communities.

4.0 Hydrology

Historical data for the site also includes hydrological patterns of drying and inundation, spanning from 1995, shortly after the ponds construction, through to 2010. The length of time that individual ponds dried out during summer months or were joined together by winter inundation across the site was recorded as days. The site was visited throughout the year and ponds recorded as inundated (i.e. overflowing sufficiently to connect with other ponds) or dry (no standing water). Times of drying and flooding were measurable to within at least 3 days, sometimes more precisely. Figure II.12 shows the extent of pond drying or inundation of the 30 ponds per month from 1995 to 2010, measured as the mean number of days that ponds were categorised under either status, alongside rainfall recorded at the local Meteorological Office in Boulmer (Ordnance survey NU 253 142, 11.9 km from the Hauxley ponds.

Figure II.12: Pond hydroperiod and rainfall recorded across the study site from 1995 to 2010.



During the study period, considerable variation in local hydrology was observed. In most years the ponds dried out in the summer, although, the precise duration of time they remained dry varied from days to weeks. Date of initial drying can also vary from late April to August. 1995 and 1996 were the end of a period, often referred to as the early 1990s UK drought (Gibbins and Heslop, 1998), whilst in 1997 heavy summer rainfall prevented summer drying, resulting in sustained inundation across the ponds throughout 1998 (Jeffries, 2008; Wheeler 1999). Water levels within the ponds fall quite gradually as they dry out; however, refill can occur rapidly, within a day during periods of heavy, sustained rainfall.

Jeffries (2010) examined the relationship between summer dry-phase and local climate for the ponds at Hauxley. Data on the length of summer dry-phase was utilised alongside climate measurements from Boulmer, including temperature, rainfall, windspeed and sunshine, to create a model of monthly variations in dry phase from January 1995 to October 2007. A model was created using two PCA axes, which captured 47% of the variation in length of monthly dry-phase. As the ponds are dependent on rainfall for recharge, this is a relatively low level of prediction. This may be hindered somewhat by the rather course time scale of month by month, which fails to capture dynamic changes in hydrology occurring over shorter timescales during periods of heavy rainfall. The relatively simplistic measures of recording the ponds status as dry or holding water also fails to take into account falling water level during periods of pond drying. However, the model does demonstrate the relationship between local weather, pond hydrology and variability between both years and months (Jeffries, 2010).

More recent studies, examining the relationship of local rainfall and incidence or area of ponds across a larger area, further demonstrate the reliance of ponds on precipitation (Jeffries, 2015). Different types of ponds displayed variable relationships with monthly rainfall. Wetlands and dune slacks showed a strong relationship over the proceeding 4 to 6 months, whereas the area of ponds in arable or pasture fields varied more with rainfall in the previous month. Results support evidence that the number and area of ponds, varies significantly both seasonally and annually, and that different pond types within different land-uses, may vary in their response to local climatic variations (Jeffries, 2015).

5.0 Newly Constructed Monitoring Ponds

In winter 2012/2013 three new ponds were constructed by the author at the Hauxley experimental site (figure II.5: ST1, ST2 and ST3). These ponds form the basis of chapter V. The aim of their construction was to monitor spatial variations in physicochemical dynamics in relation to primary productivity and OC burial across the early stage of succession. The ponds have been monitored at approximately fortnightly intervals from April 2013 for various parameters including depth, conductivity, nutrients and chlorophyll-*a.*

6.0 Carbon burial

In 2012, the ponds formed the basis of research exploring their effectiveness at sequestering and storing OC. The known construction date of the ponds coupled with comprehensive historical data on hydrology and vegetation community succession provides a unique opportunity to produce OC burial rates and assess dominant factors driving this important ecosystem function. The study sampled ten of the ponds at Hauxley, results from which have been included as part of a wider publication by Gilbert et al., (2014), assessing variations in sediment OC between different types of small natural ponds at Druiridge Bay.

The study was exploratory. Pole corers with a 4cm internal diameter were used to ensure penetration of original compacted bottom soil. Samples were air dried for >4 days before dissection. Cores were separated into 3 distinct layers based on visible laminations, consisting of a top layer of sediment, an underlying transitional layer, and the undisturbed pond bottom, as described by Munsiri et al., (1995). Cores contained >5% OC in the top layers and displayed a decrease in OC with depth to ~5cm, at which point OC content becomes indistinguishable from the surrounding soils. Distinct separation of the cores above and below this ~5cm depth point represents a clearly visible boundary between organic-rich sediment and underlying clay soil. Knowledge on the construction date of the ponds allows OC burial rates to be estimated based on OC storage values within the top sediment layers and also include the underlying transition layer. Results from which can be seen in table II.3.

OC burial rates in the ponds were estimated to be around 91.3 (51 – 139) g OC m^{-2} yr^{-1} for upper sediment layers, This increased to 149 (108 – 173) g OC m^{-2} yr^{-1} with the inclusion of an underlying transition layer (Gilbert et al., 2014). Burial rates were substantial in comparison to other natural aquatic and terrestrial ecosystems (Downing et al., 2008, Duarte et al., 2004; Schlesinger, 1997; Mulholland and Elwood, 1982).

Table II.3: Carbon storage estimates from an undergraduate study carried out at the site.

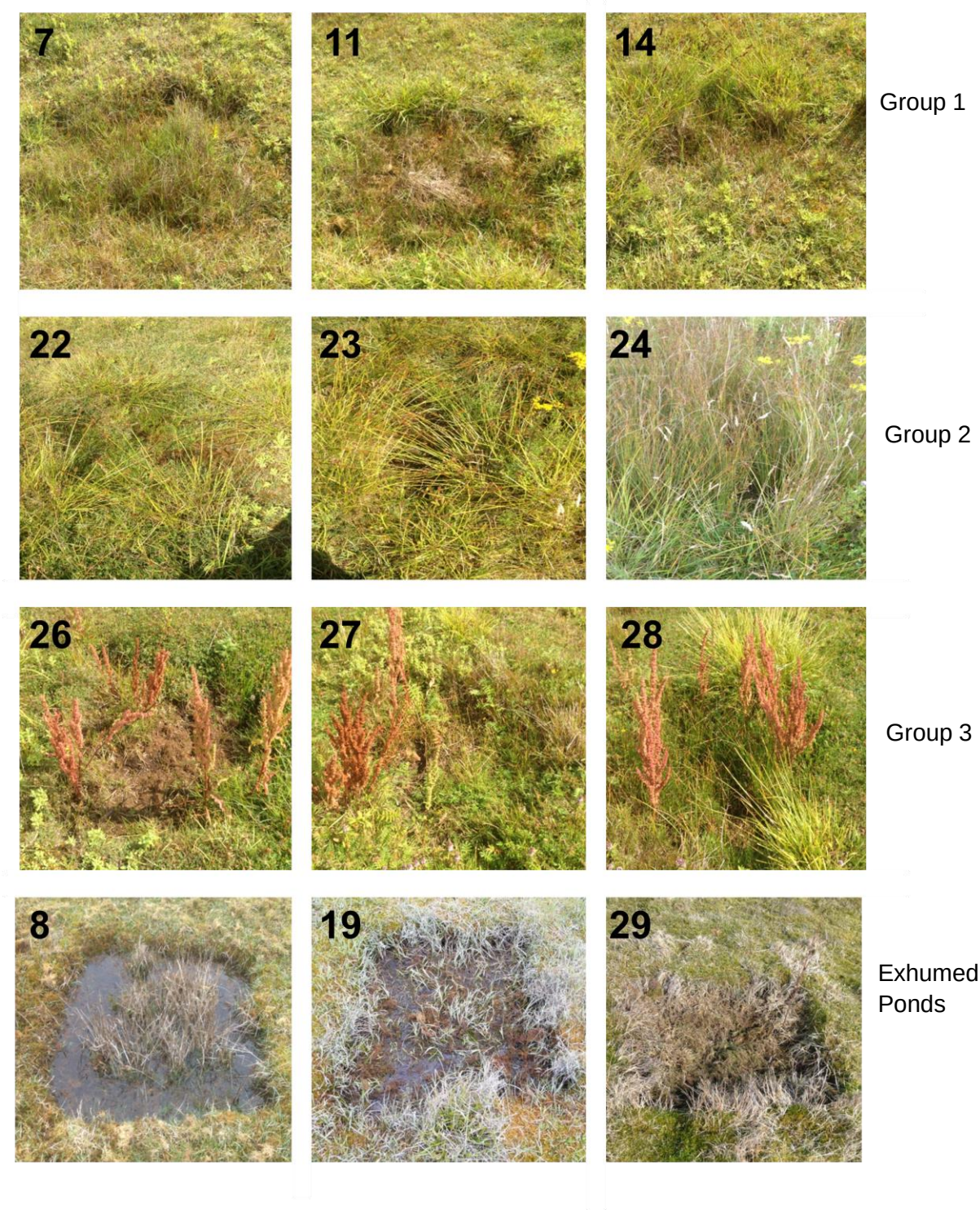
	Top Layer		Top and Middle layer	
Pond	OC Stored (g)	OC Burial (g OC m^{-2} yr^{-1})	OC Stored (g)	OC Burial (g OC m^{-2} yr^{-1})
P30	1721.68	95.65	2638.22	146.57
P1	1948.09	108.23	2521.18	140.07
P2	2500.32	138.91	3113.96	173.00
P3	1704.38	94.69	2598.41	144.36
P4	1954.44	108.58	3120.00	173.33
P5	1269.05	70.50	2660.67	147.82
P10	1644.10	91.34	2185.60	121.42
P13	917.15	50.95	1937.92	107.66
P17	1191.42	66.19	3012.04	167.34
P21	1583.39	87.97	3084.03	171.33
Mean	1643.40	91.30	2687.20	149.29

Extensive spatial variation was also observed between ponds. Ponds in the southern area of the site displayed higher burial rates than those in the north, surprising given the relatively short overall distance between the ponds, same rainfall and same nutrient inputs (Gilbert et al., 2014). The 3 ponds containing the highest OC% have been dominated by thick swards of *L.riparium* and aquatic grasses since the late 1990s in comparison to ponds that retained a more open flora of *C.vulgaris* and *R.aquatilis*.

7.0 Pond Selection for Chapters IV and VI

For this study, the ponds were split into three distinct groups representing past vegetation community succession. For chapter IV one pond was taken from each group; Group 1 – pond 8, Group 2 – pond 19, Group 3 – pond 29. In chapter VI a triplicate of ponds was chosen for each zone; ponds 26, 27 and 28 (Group 3) represented ponds that displayed an early establishment of *L.riparium*, whilst ponds 7, 11 and 14 (Group 1) represented ponds that supported more aquatic based vegetation due to *L.riparium* taking longer to establish in these ponds. Ponds 22, 23 and 24 (Group 2) were included as an intermediate set of ponds along the pathway of *L.riparium* establishment, but also represent ponds displaying earlier establishment of *J.articulatus*. Ponds selected can be seen in figure II.13.

Figure II.13: Ponds selected for chapters IV and VI representing distinct groups based on past vegetation community succession.



Chapter III: Methods & Methodological Development

1.0 Sediment Cores and Subsequent Analysis

1.1 Sample Collection and Corer Development

The requirements of sediment cores are to recover an undisturbed sample that includes the sediment/water interface. Hvorslev (1949) outlined the following requirements for an undisturbed sediment core; i) no disturbance of structure ii) no change in water content or void ratio iii) no change in constituent or chemical composition (Glew, Smol & Last, 2001). Aquatic sediments can vary greatly in density and consistency within individual bodies and between different types. A number of significant factors impact sediment composition, such as pathways of material transport to the water body, transport through the water column and diagenetic alteration as it is incorporated into the sediment (Glew, Smol & Last, 2001).

Retrieving representative cores can be difficult and will always be considered by some as a rather unpredictable 'grey area' of research involving collection and extrusion of sediment cores. The retrieval of adequate samples is further complicated by uncertainty such as water depth and sediment consistency (i.e. is it consolidated or unconsolidated with high moisture content as can be the case in anoxic environments). New coring devices and modifications of existing coring apparatus are constantly being developed, however, no one type of sediment corer is applicable to all types of studies and conditions (Glew, Smol & Last 2001).

1.1.1 Problems encountered with sediment core collection

Numerous problems were encountered in the early developmental stages of this research in regards to collecting sediment core samples. Key problems were:

1) Water Depth – Depth in the ponds sampled ranged from 0 - 70 cm. How can intact sediment cores be retrieved from beneath 70cm water?

2) Sediment Density and Composition – Sediment composition was markedly different between ponds due to hydrology (e.g. temporary ponds with dried desiccated sediments were difficult to penetrate) and land use (e.g. underlying clay soils were much denser than sandy substrates in dune slack ponds).

3) Vegetation Type – The type and amount of flora covering the surface of many ponds varied considerably, from algal mats, thick moss swards and dense root mats associated with species such as *Phragmites.* Problems were encountered trying to penetrate these dense layers and extruding cores, without disturbing the pond sediment.

4) Calculation of Sediment Dry Bulk Density – DBD is a cucial measurement in the estimation of carbon stock and density. As results are extrapolated to cover the whole system It is critical that values obtained are as accurate and reproducible as possible to allow accurate comparison between different ponds.

5) Discrepancies in Results from Different Coring Techniques – Different coring techniques were used to overcome the aforementioned problems (see table III.1), but discrepancies in OC% observed between different sampling techniques were observed leading to uncertainty as to whether such variations were driven by sediment composition or sampling technique .

Table III.1: Coring techniques used and associated problems encountered.

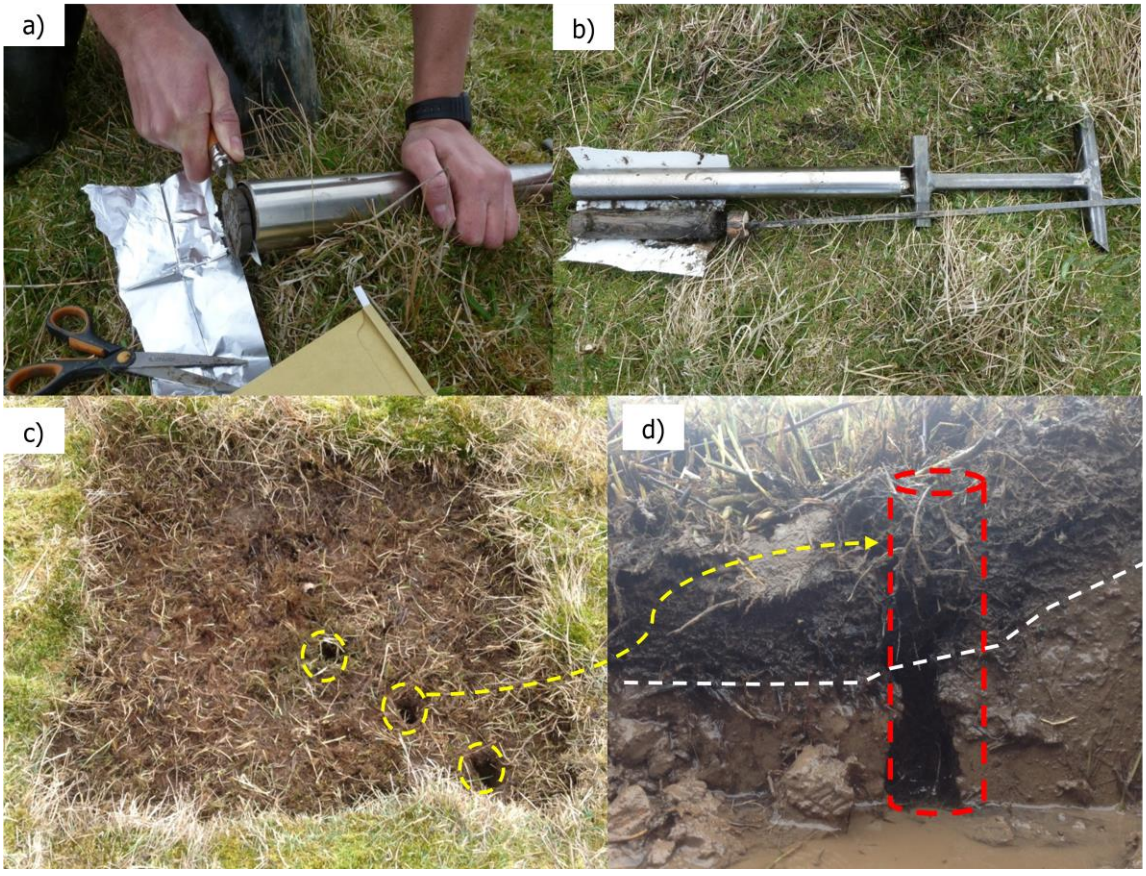
Coring Method	Problems Encountered
Plastic Pole Coring	• 1.25” diameter tubing yielded Insufficient material for analytical testing • 2.5” diameter tubing struggles to penetrate root mats and denser sediments, • Blunt base failed to penetrate denser substrates to any significant depth • Extrusion of sediments with plunger and dissection at 1cm resolution difficult for softer sediments, due to high levels of friction and compaction • Drying the core prior to extrusion was trialled but also presented problems in calculating dry bulk density, dissecting the core accurately and sample loss from dust produced when cutting
Open faced metal corer (Russian peat corers)	• Sharpened edge allowed penetration of sediments to greater depth. • Open faced allowed ease of access to sediment cores for dissection. • Useful for denser sediments but looser and wetter sediments would fall out the open face.
Dry ice cores	• Low density sediments hard to extract with plastic tube cores and the open faced core • Freeze core extraction facilitated collection of waterlogged and unconsolidated sediments • Laminations kept intact as they are frozen to outside of the corer • Dissection and separation difficult

The development of a robust reproducible coring method was a necessity for this project and the research group as a whole, allowing results from different researchers and ponds to be compared with accuracy. Several prototypes were developed and trialled to identify a model that alleviates problems observed with other techniques. Larger corers struggled to penetrate the often dense mats of vegetation in reed beds of heavily vegetated ponds, whilst shorter corers failed to reach the soil base layer required to “plug” accumulated pond sediment in the core.

The final design was made using Chromium-vanadium steel (High Polish 2P). The 2P finish has minimal micro crevices, which optimises corrosion resistance and greatly reduces friction between the corer and sediments. The bottom of the core was given a bevelled cutting edge, which was filed and sharpened to facilitate effective penetration of desiccated sediments and dense root layer or vegetation mats. An internal graduated plunger was added to allow efficient extrusion of sediment material, whilst providing an accurate gauge of compaction.

The final design seen in figure III.1b facilitates the sampling of pond sediment varying in structural integrity, and provides an efficient extrusion tool marked with points at 1cm increments to dissect cores at standardised intervals. Samples can be dissected quickly and effectively in the field and placed directly into pre-weighed foil and sample bags for transport back to the labs. Samples can then remain in the bags throughout the drying process minimising cross contamination and loss of material. Sediment core positions and dissection method can also be seen in figure III.1c and III.1a, as can the core profile consisting of upper sediment layers and the base clay layer III.1d.

Fig III.1: a) Sediment corer, sediment core and extrusion tool. B) Dissection of sediment into preweighed foil and sample bags. c) Sediment cores taken from an exhumed pond Ch.IV. d) Cross sectional view of sediment core showing distinct sediment-clay interface.



1.2 Measurement of sediment properties

1.2.1 Moisture content

Samples were weighed within 24hrs of coring to identify the wet weight of individual core sections. Samples were subsequently placed in a drying cabinet at 40°C for ~7days and the dry weight was recorded upon the sample reaching a constant weight. Moisture content (% moisture) was then calculated using the following equation.

$$\%\text{moisture content} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} * 100$$

1.2.2 Dry bulk density

Dry bulk density (DBD) values are essential components of mass accumulation rate calculations and estimates of C density and OC storage, Mass accumulation rates incorporate the effects of depositional and post-depositional processes such as compaction and diagenetic alteration, as well as sediment composition (Dadey et al., 1992). DBD is defined as the mass (weight) of the dry solids divided by total volume of the wet sample; that is DBD is the ratio of the mass of soil/sediment to the total volume:

$$\text{Dry Bulk Density (g cm}^{-3}) = \frac{\text{Dry Weight (g)}}{\text{Wet Volume (cm}^3)}$$

1.3 Grinding and Sieving

Dried samples were ground using a pestle and mortar, removing larger pieces of material such as twigs, roots and stones. Samples were then sieved at 0.5 µm. All equipment was cleaned with acetone between samples to avoid cross contamination. The prepared samples were then sealed in 10 mL vials and frozen (<4°C) prior to analysis.

1.4 Analysis

1.4.1 Inorganic carbon (IOC)

Inorganic carbon forms are derived from geologic or soil parent material sources, although may form as a result of precipitation in alkaline environments. Inorganic carbon in soils and sediments usually consists of carbonates such as calcite $CaCO_3$ and dolomite [$CaMg(CO_3)_2$]. Elemental analysis (EA) provides a result for total carbon (TC) which includes both organic (OC) and inorganic (IOC) fractions. As it is primarily

OC upon which this study is focused, it is important to ascertain the proportion of TC that may be comprised of IOC.

Hydrochloric acid (HCl) digestion was used to determine IOC concentrations within sediments of the ponds. Three air dried samples were selected and subject to acid digestion. Roughly 10 g of sample was placed in a pre-weighed beaker and flushed with (HCl). Samples were observed for any effervescence, indicating the presence of carbonates, prior to drying at ~60°C for 24h. Samples were then weighed and the percentage loss calculated to indicate levels of IOC.

Results from the HCl digestion (see table III.2) suggest IOC concentrations within the Hauxley pond sediments are minimal, with a mean IOC% of 0.032 (0.008 – 0.056). Extrapolations to an entire pond using the total accumulated weight of sediment from Pond 19 in chapter IV (13437.3 g) would suggest that IOC comprises of 4.25 g of total sediment weight as oppose to 1676.96 g observed for the OC. In comparison to OC% results from loss on ignition (LOI) and elemental analysis (EA), these concentrations are substantially less, suggesting that the inorganic fraction is negligible.

Given the surrounding landscape and underlying geology, this is of no surprise, as inorganic carbonates are usually found in areas with underlying limestone geology or alkaline aquatic environments, where precipitation of carbonates can be a significant process.

Table III.2: Results inorganic carbon analysis via HCl digestion.

Inorganic Carbon Analysis				
Sample	Total Weight (g)	Weight after HCl digestion (g)	% Loss	% Inorganic carbon
P19.1	115.8632	115.798	0.05627	0.0563
P19.2	113.2267	113.192	0.03064	0.0306
P19.3	106.6344	106.626	0.00787	0.0079
			Mean	**0.032**

1.4.2 Carbon analysis LOI-TEA calibration

Organic Carbon (OC)

Naturally occurring OC forms are derivatives from the decomposition of plants and animals. In sediments and soils this may take the form of freshly deposited litter (e.g. twigs, leaves and branches) right through to highly decomposed humic material (Schumacher, 1998).

Loss on Ignition (LOI) is a commonly used method to determine the OC content of soil and sediment samples (Downing et al., 2008; Heiri et al., 2001; Schumacher 1998). The process involves the combustion of OM in a furnace usually at temperatures of around 550°C (Downing et al., 2008; Heiri et al., 2001; Schumacher, 1998). The weight loss difference of a sample is taken to be the OM content. Methods used in terms of furnace temperatures, duration of ignition and conversion factors used to convert OM to OC varied considerably across the literature. Heiri (2001) indicated that factors such as the aforementioned, including position of the crucibles in the furnace and sample size might have a considerable influence on results from the LOI.

It was also stated that ignition temperatures should be kept below 550°C as they will commonly result in underestimation of OM content (Boyle, 2003). This contradicts Schumacher (1998) who states that temperatures should be maintained below 440°C to avoid the destruction of any inorganic carbonates that may be present, and that the loss of structural water from clay minerals should also be considered when interpreting results.

There has also been substantial variability in the conversion factors used to convert OM content to OC. The most commonly used conversion factor is 1.72 based on the assumption that OM contains around 58% OC (Nelson & Sommers, 1996). However due to variations between soil types, soil horizons and within individual soils, there is no universal conversion factor. Factors range from 1.72 to as high as 2.5 (Nelson & Sommers, 1996; Soil Survey Laboratory Methods Manual, 1992), with 1.9 and 2.5 being used as a conversion factor for surface and subsurface soils respectively (Schumacher,1998).

To assess the accuracy in using this method LOI was performed on samples varying in OC%, as determined by elemental analysis (EA), in an attempt to calibrate our own temperature, time and conversion factor. Two samples were selected, one from an arable pond with low OC% and one from a naturally vegetated pond with high OC%.

Samples were subject to LOI at a range of temperatures from 350°C, in 50°C increments up to 600°C, at durations of 2, 4 and 6 hours at each temperature. For each sample, there were 18 results for OM% that were used to calibrate against results observed from EA analysis on the same samples, using a range of OC% conversion factors (see table III.3). Results from the calibration did not fit as previously anticipated as optimum duration and conversion factors varied between the samples at a temperature of 350°C, much less than temperatures recommended in the literature (Downing et al., 2008; Heiri et al. 2001). Due to these results, it was decided that further OC% analysis will be carried out by EA.

Table III.3: Results from the LOI-EA calibration. Values Highlighted in red indicate values matching closest to results from EA analysis

Pond Type		Arable Pond				Naturally Vegetated			
Temp	Duration	OM	OC% (Conversion Factor) (1.72)	(1.9)	(2.5)	OM	OC% (Conversion Factor) (1.72)	(1.9)	(2.5)
350	2	3.39	**1.97**	1.78	1.35	27.86	16.20	14.66	11.14
	4	3.78	2.20	1.99	1.51	29.91	17.39	15.74	11.96
	6	3.78	2.20	1.99	1.51	29.64	17.23	**15.60**	11.86
400	2	3.35	1.95	1.76	1.34	30.06	17.48	15.82	12.02
	4	4.12	2.39	2.17	1.65	32.22	18.73	16.96	12.89
	6	4.04	2.35	2.13	1.62	30.85	17.94	16.24	12.34
450	2	4.33	2.52	2.28	1.73	30.36	17.65	15.98	12.15
	4	5.75	3.35	3.03	2.30	33.56	19.51	17.66	13.42
	6	5.75	3.34	3.03	2.30	32.90	19.13	17.32	13.16
500	2	5.32	3.09	2.80	2.13	33.20	19.30	17.48	13.28
	4	6.60	3.84	3.48	2.64	34.12	19.84	17.96	13.65
	6	6.40	3.72	3.37	2.56	33.99	19.76	17.89	13.59
550	2	6.69	3.89	3.52	2.68	33.34	19.38	17.55	13.34
	4	6.98	4.06	3.67	2.79	34.45	20.03	18.13	13.78
	6	7.26	4.22	3.82	2.90	34.05	19.80	17.92	13.62
600	2	6.69	3.89	3.52	2.68	33.83	19.67	17.80	13.53
	4	6.91	4.02	3.64	2.76	34.19	19.88	17.99	13.68
	6	7.57	4.40	3.98	3.03	33.98	19.76	17.89	13.59
TEA Result		**1.97**				**15.2**			

1.4.3 Carbon and nitrogen analysis

Elemental analysis (EA) is a method for the rapid determination of carbon, hydrogen, nitrogen and sulphur in organic matrices and materials. EA involves high temperature combustion in an oxygen rich environment under static conditions (i.e. set volume of gas) or dynamic conditions (i.e. constant flow of gas for a set time period). In the combustion process, carbon is converted to carbon dioxide; hydrogen to water; nitrogen to nitrogen gas/oxides and sulphur to sulphur dioxide. Combustion products are swept out of the combustion chamber by an inert carrier gas such as helium and passed over heated high purity copper. Copper removes any oxygen not consumed in the combustion process and converts oxides of nitrogen to nitrogen gas. Detection of these gases is carried out by quantification using thermal conductivity detection, after calibration with high purity micro analytical standards, such as aspartic acid.

Samples were analysed on a Thermo Scientific Flash 2000 Organic Elemental Analyser configured to determine CN. ~5 mg of sample was placed in tin caps prior to entry into the autosampler. Initial combustion was carried out at 980°C, and the heated copper furnace at 680°C with an ambient oven temp 50°C. Helium was used as a carrier gas and was set at a flow rate of 130 mL/min and oxygen at 250 mL/min. Cycle duration was set to 360 seconds with a sample delay of 12 seconds and an Oxygen injection to flush the system at 355 seconds.

1.4.4 Data validation

As a quality control measure in EA analysis, every 10th samples was run in triplicate, followed by a blank sample (n=44). Blanks were used to identify values for LoD (Limit of Detection) and LoQ (Limit of Quantification), with triplicate samples being used to determine the precision of the analysis (%RSD; %Relative Standard Deviation). The equations used to calculate the aforementioned values are displayed below.

$$LoD = Mean_{blanks} + (3 * \text{standard deviation}_{blanks})$$

$$LoQ = Mean_{blanks} + (10 * \text{standard deviation}_{blanks})$$

$$\%RSD = \frac{\text{standard deviation}_{\text{triplicates}}}{\text{Mean}_{\text{triplicates}}} * 100$$

LoD was calculated to be 0.53 %C and the LoQ was 1.64 %C. Triplicates were comprised of a wide range of samples including different ponds and various sediment depth layers. %RSD averaged 8.94 %RSD with the majority of samples being under <10 %RSD and only 4 were >15 %RSD.

1.4.5 Determination of sediment clay-Interface - ED-XRF analysis

Energy Dispersive X-ray Fluorescence (XRF) Analysis has been used to identify the sediment-clay interface (i.e. the point at which accumulated sediment stops and the original clay bottom soil begins). Identifying this boundary layer with precision is critical if accurate estimates of OC storage and accumulation rates are to be produced.

Clay is composed mainly of silica, alumina and water, often alongside appreciable quantities of iron, alkali metals and alkali earth metals (Nayak and Singh, 2007; Ralph, 1986). XRF analysis was used to analyse concentrations of aluminium, silicon, magnesium, potassium and iron to use as variables representing the clay soil layers, alongside phosphorus and sulphur to represent the sediment fraction.

Samples were analysed as pellets made up of ~4 g of sample and ~0.7 g of FluXana CEREOX Licowax binder. Pellets were analysed in a Spectro X-lab 2000. A correction factor was applied to results post analysis, following calibration against reference soils with known metal concentrations. Reference soils covered a range of concentrations and linear equations were used to determine an appropriate correction factor.

DCA analysis was performed alongside other physicochemical variables to separate samples by sediment and clay. Full details of the methods used to determine the sediment-clay interface will be discussed in the following chapter (IV).

1.5 Carbon Quantification

1.5.1 C concentration – OC%

Output from the EA analysis is a % value of C and N, calculated directly from the individual weight of the sample being analysed. This value therefore represents the OC% within the particulate matter of the sediment sample.

1.5.2 C density – mg OC cm^{-3}

The OC% of an individual sample provides insights into the OM content within the sediment; however, it is important to quantify the mass or C density, within each sedimentary layer relative to the overall dry mass.

C density was calculated by factoring in sediment DBD using the equation outlined below.

$$\text{C density (mg OC cm}^{-3}) = \frac{\text{DBD}}{100} * \text{ \%OC (*1000)}$$

1.5.3 Carbon storage – g OC m^{-2}

Estimating whole pond OC storage requires extrapolation of values obtained from sediment core analysis. This is done by; 1) determining the sediment-soil boundary, 2) quantifying the absolute mass of carbon stored in the sediment profile within the core and 3) extrapolating this value over a m^2 area.

$$\underset{\text{(g OC)}}{\text{Carbon stock per sediment section}} = \underset{\text{(mg OC cm}^{-3})}{\text{C density}} \times \underset{\text{(cm}^{-3})}{\text{volume of sediment layer}}$$

$$\underset{\text{(g OC)}}{\text{Carbon stored in sediment core}} = \text{Sum of carbon stock values in identified sediment layers } \underset{\text{(g OC)}}{}$$

$$\text{Carbon Storage g OC m}^{-2} = \frac{\text{Carbon stored in sediment core (g OC)}}{\text{Surface area of corer (cm}^2)} *10000$$

Carbon Burial Rate = Carbon Stored (g)/Age of pond (yrs)

See Chapter IV for methods on determining the sediment-clay interface.

See Appendix for a worked example of sediment core extrapolation.

1.6 Nitrogen

EA analysis also provides analysis of Nitrogen concentrations (%N) within the sediment. LoD and LoQ for Nitrogen was calculated as 0.077% and 0.25% respectively. %RSD was on average 16.60% and displayed considerable variation between ponds and down the sediment profile.

1.7 Carbon:Nitrogen ratios

C and N can be compared as a ratio to provide a potential indication as to the composition and source of OM within sediments (Meyers and Ishiwatari, 1993). C:N ratios have been calculated using the following equation:

$$\text{C:N ratio} = \frac{\text{\% C}}{\text{\% N}}$$

1.8 Statistical Analysis

1.8.1 Repeated measures ANOVA

ANOVA is a powerful statistical model designed for testing statistical significance among the means of two or more groups. It has a practical application in this study allowing for direct comparisons of the mean of a dependant variable (e.g. DBD, OC% and C density) against pond groups, whilst still maintaining individual subjects (individual ponds).

ANOVA models allow the inclusion of *repeat measures* into the analysis. Several measurements from individual subjects (e.g. C density of individual 1cm slices down a core) are repeat measures as opposed to wholly independent measurements and are therefore likely to be more similar to one another. Another useful tool is the application of a covariance structure on repeat measurements. In this study, repeat measurements from sediment cores, lend themselves to an autoregressive [1] (AR[1]) covariance structure. This assumes that adjacent measurements are more closely correlated than those that are further apart; i.e. sediment layers at depths 1 and 2 are more closely correlated than those at 1 and 10cm depths.

Factors in this model can be run as fixed (i.e. it assumes all potential groupings have been sampled) or random factors (i.e. the model assumes individual ponds sampled are a random sample of all ponds across the landscape). This facilitates the development of a more appropriate model. Numerous methods exist for developing the best model (e.g. set up of random factors and co-variables), all models in this thesis were selected to be the most efficient; i.e. the model which provides the greatest level of information for the lowest number of parameters.

Although ANOVA tests for statistical significance amongst groups of means, it does not state where those differences lie. A post analysis Bon-Ferroni test was applied to all ANOVA models, which carries out pairwise comparisons among groups within the fixed factor, stating the statistical significance observed between them. ANOVA was performed in SPSS statistical software package.

2.0 Physicochemistry and Productivity

2.1 Sample collection and storage

Chemical

Water samples were collected in acid washed polyethylene bottles. For each pond 500 mL of water was collected from each of the 4 corners and 2 points were taken from the centre, all samples were taken at various depths in the pond creating a homogenous sample (i.e. a one sample composite per pond). Samples were stored in a cool box under darkened conditions at 4°C and taken back to the lab. Samples were subsequently stored at 4°C for up to 48 hours prior to analysis.

Chlorophyll-*a*

From April 2013 - March 2014 100 mL of water was collected from various points within the pond, creating a homogenous sample. Samples were then passed through 0.45 µm Whatman gf/f filters. Filters were stored in ice, under darkened conditions and transported back to the laboratory before subsequent storage at -80°C prior to chlorophyll-*a* analysis. From March 2014 - February 2015, 500 mL samples of homogenised water were collected in acid washed polyethylene bottles. Samples were stored under ice and darkened conditions, prior to immediate analysis upon return to the lab.

2.2 Analysis

2.2.1 Basic physicochemical variables

April 13 – March 14. Conductivity was measured with a HANNA instruments HI 9033 multi range conductivity meter and dissolved oxygen via colorimetric analysis with Chemetrics self-filling ampoules, compared against Chemetric standards. pH was measured in the laboratory using a Jenway 3505 pH meter.

March 14 – April 16. Conductivity, dissolved oxygen, pH and temperature parameters were analysed using a Macro 900 Meter fitted with (MAP) 2000 macro accessory probe. This instrument was calibrated to the same standards as previously mentioned.

2.2.2 Nitrates NO_3^--N

NO_3^--N was determined using the cadmium reduction method. Samples were analysed using a Hach DR2800 Spectrophotometer at 500nm.

2.2.3 Phosphates PO_4^{3-}

PO_4^{3-} was determined using the Ascorbic Acid method. Samples were analysed using a Hach DR2800 Spectrophotometer at 880nm.

2.2.4 Ammonia NH_3

NH_3 was measured using the indophenols method. Ammonia reacts with alkaline salicylate in the presence of chlorine to form a green-blue indophenols complex, which can be measured spectrophotometrically.

2.2.5 Nitrite NO_2^-

Nitrite concentrations were determined via reaction with sulphanillic acid in an acid solution. Resulting diazo compound couples with N-(1-napthyl)-ethylene diamine to form a reddish dye, which can be measured spectrophotometrically.

2.2.6 Turbidity

Turbidity samples were analysed using a Hach DR/2010 Spectrophotometer at 860nm.

2.2.7 Chlorophyll-a

Chlorophyll-*a* was measured with a Chlorophyll-*a* optical probe, fixed response fluorometer fitted to the Macro 900 MAP 2000. The probe provides excitation at 470nm inducing chlorophyll-*a* to fluoresce, the chlorophyll-*a* then emits longer wavelengths as part of the fluorescent process and the probe detects any resultant fluorescence above 630nm.

Samples taken April 2013 to March 2014, had filter membranes extracted and placed in 100 mL deionized water. These filter samples were sonicated, under darkened conditions, for 10 minutes prior to analysis with the optical probe. For samples taken March 2014 to April 2016, 500 mL of water was collected in acid washed polyethylene bottles and stored under ice and darkened conditions for transport back to the laboratory. A triplicate of subsamples were taken and sonicated, in darkened conditions, for 10 minutes and analysed with the Macro 900 meter.

2.3 Chlorophyll-*a* Analysis

The analysis of chlorophyll-*a* concentrations within a waterbody is a well established marker for primary productivity (Gibb et al., 2001; Crisman et al., 1998). Chlorophyll-*a* was analysed with a palintest Macro 900 PT 1556 Chlorophyll-*a* optical probe, a submersible fixed response fluorometer, which provides excitation at 470nm and

detects any resultant fluorescence above 630nm. The probe induces chlorophyll-*a* to fluoresce, and then measures the longer wavelength light which is emitted as a result of the fluorescence process.

Determination of chlorophyll-*a* concentrations in the field using fluorescence measurements has its limitations in comparison to either cell counting or analysis of molecular chlorophyll-*a*, such as HPLC, after extraction from cells. Data obtained with the probe in the field should therefore be post calibrated with data from the laboratory analysis of samples.

2.3.1 Chlorophyll-a probe calibration

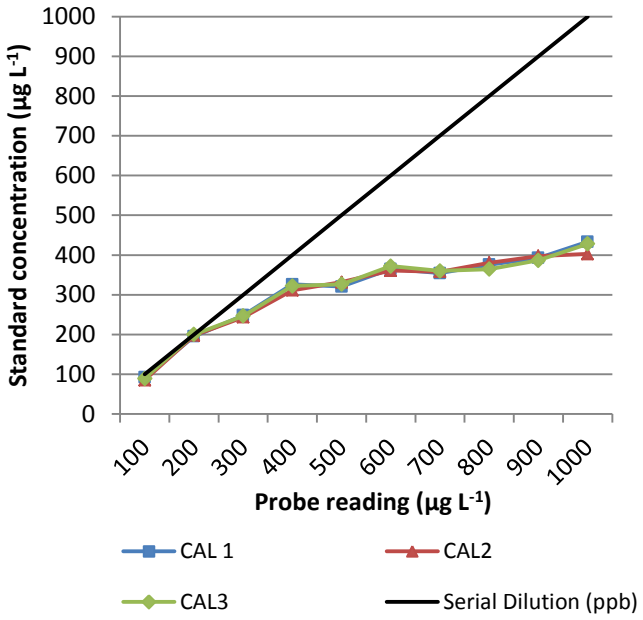
The use of molecular techniques, such as HPLC, or spectrophotometrical techniques to quantify chlorophyll-*a* concentrations were beyond the scope of this study. Although industry (ISO) recognised, these techniques are most often used for the taxonomic classification of phytoplankton species and pigments, as opposed to general concentrations (Descy et al., 2008; Li et al., 2003). Studies have identified that results from submersible fluorescense probes can correlate highly with standard ISO methods and that such techniques are useful for in-situ sampling (Gregor & Maršálek, 2004). The objective of this study was to obtain measurements of the primary productivity of the ponds in relation to physicochemistry and explore its effect on OC burial. In order to assess probe accuracy, calibrations were carried out using chlorophyll-*a* standards. The accuracy of methods for taking in-situ measurements were also assessed.

Standards were made by extracting chlorophyll-*a* from spinach leaves. Dikio and Isabirye (2008) reported a yield of 23 - 24 mg of chlorophyll-*a* from 100 g of spinach leaves. Stock solutions at concentrations of 1 mg L^{-1} were made using 4.25 g of spinach leaves which would yield 1 mg of chlorophyll-*a* according to Dikio and Isabirye (2008). Leaves were ground and flushed with small amounts of acetone in order to promote the suspension of chlorophyll-*a* in solution. Samples were then added to 1 L of deionised water to make a stock solution of 1 mg L^{-1}. The final solution was sonicated for ~5 mins to release chlorophyll-*a* from the remainder of any leaf fragments. Standards were subsequently validated against purchased standards (Sigma Standard CS144 Chlorophyll-a) and displayed an error of 8-10% across the 10 – 150 μg L^{-1} range.

Serial dilutions were made from stock solutions to cover a range of resolutions in order to assess the probes relative sensitivity at various chlorophyll-*a* concentrations. A triplicate of stock solutions were made and a subsequent triplicate made for each serial

dilution. Ten probe readings were taken from each serial dilution in order to produce an overall mean value. The first experiment assessed probe accuracy across the 100 – 1000 μg L^{-1} range in 100 μg L^{-1} increments. Results can be seen in figure III.2**.** Results suggest a substantial range in percentage error across this resolution (1.18% - 57.87%) and that the probe remains relatively accurate to around 200 μg L^{-1} (table III.4). Further incremental increases deviate substantially from the standard concentrations and increases in associated percentage error can also be observed.

Fig III.2 and Table III.4: Chlorophyll-*a* probe calibration across 0 – 1000 μg L^{-1} range.



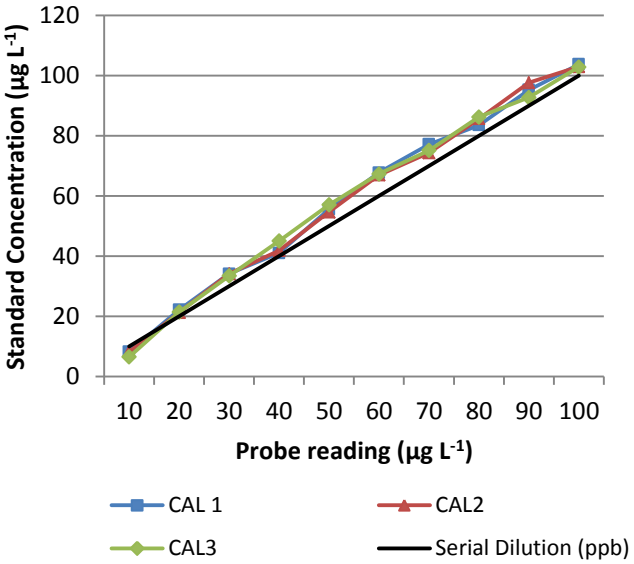
Serial Dilution (μg L^{-1})	Mean	% error
100	88.83	11.17
200	197.63	1.18
300	246.13	17.96
400	319.83	20.04
500	326.33	34.73
600	365.77	39.04
700	357.53	48.92
800	373.43	53.32
900	392.17	56.43
1000	421.33	57.87
Mean % error		34.07

Results suggest a maximum threshold reading of around 400 μg L^{-1}, although associated % error at these concentrations is substantial (20.04%) and indicates that readings obtained at this threshold should be reproduced with caution. A more reasonable and reproducible maximum threshold would be around 200 μg L^{-1} at which point %error is minimal at 1.18%, although factoring inaccuracy at the 100 μg L^{-1}threshold (11.17%) would give a mean percentage error across these concentrations at 6.18%. Given that chlorophyll-*a* concentrations >40 μg L^{-1} are classified as hyper-eutrophic it is unlikely that chlorophyll-*a* concentrations observed across the study would surpass the maximum threshold of 200 μg L^{-1}.

A second experiment focused on smaller resolutions across the 0 -100 μg L^{-1} range at increments of 10 μg L^{-1}, and 0 – 150 μg L^{-1} range at increments of 25 μg L^{-1}, in order to assess the relative sensitivity and reproducibility of readings at smaller resolutions

more likely to be encountered across the study period. Results can be seen in figure III.4 whilst the associated percentage error can viewed in table III.5. Results indicates a greater degree of accuracy across this resolution, which displayed much smaller range in percentage error (3.13% – 23.67%), with the greatest degree of error associated with concentrations at the lowest threshold (10 µg L^{-1} – 26.67%) and mid threshold values (50 µg L^{-1} – 11.4%, 60 µg L^{-1} – 12.17%). The overall % error associated with this resolution is around 9.76%, which was deemed acceptable for the reproducibility of results across a sustained monitoring period.

Fig III.3 and Table III.5: Chlorophyll-a probe calibration across 0 – 100 µg L^{-1} range.



Serial Dilution (µg L^{-1})	Mean	%error
10	7.63	23.67
20	21.57	7.83
30	33.87	12.89
40	42.60	6.50
50	55.70	11.40
60	67.30	12.17
70	75.50	7.86
80	85.10	6.38
90	95.17	5.74
100	103.13	3.13
Mean % error		9.76

Calibration plots with associated regression equations can be seen in figure III.4. Values across the 1-100 µg L^{-1} calibration and 0-150 µg L^{-1} calibration display strong coefficient values with R^2 values of 0.995 and 0.9912 respectively. This value is reduced across the 0-500 µg L^{-1} calibration displaying an R^2 value of 0.942.

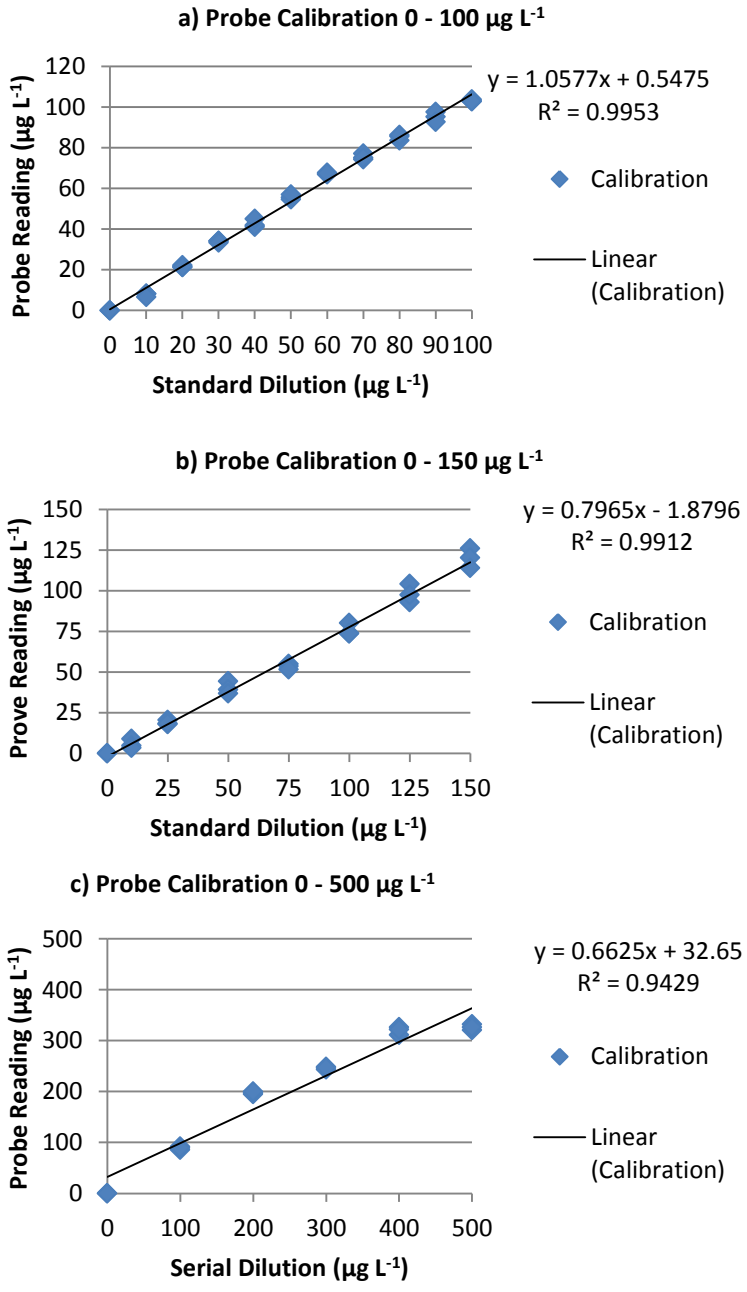
2.3.2 Chlorophyll-a probe analysis

Having assessed the probe's relative sensitivity against chlorophyll-*a* standards the next step was to assess the accuracy of in-situ sampling in the field. Initial trials of *in-situ* measurements produced highly variable results with personal observations in the range of 0 – >100 µg L^{-1} over the course a single immersion into the pond. Stability of sample readings throughout this trial was also poor with the probe often failing to stabilise in order to take accurate readings. This is likely a result of the uneven

distribution of chlorophyll-*a* within a water body where cells can often cluster together or bind to small particulates, leading to instability and variability in probe readings.

Following these trials, it became apparent that a suitable sampling protocol had to be established to overcome limitations experienced in direct in-situ sampling. Sonication is widlely recognised in methods of quantifying chlorophyll-*a* from freshwater algae, to aid the stability and reproducibility of chlorophyll-*a* readings (Simon & Helliwell, 1998). In principle the aim is to break opon algal cells to make a more homogenous solution of chlorophyll-*a*. Sonication was performed using a Sonic 6000m ultrasonic bath.

Figure III.4: Chlorophyll-*a* probe calibration plots across a range of concentrations displaying linear regression equations and coefficient values (R^2).



An experiment was set up to assess the accuracy over a range of sampling methods in triplicate over the three newly constructed experimental ponds (ST1, ST2 and ST3). In-situ samples were collected using direct probe measurements within the pond collecting ~50 readings, three 500 mL samples were then collected and stored in ice prior to sonication under darkened conditions for 10 minutes. A further three samples were filtered prior to sonication for 10 minutes, to remove particulates which may interfere with the fluorescence methods. 20 probe readings were taken for each sample that had undergone sonication. Results are displayed in figure III.5.

Figure III.5: Boxplots showing Chlorophyll-*a* readings taken with different sampling techniques. In-situ: direct immersion, S: Sonicated, SF: Filtered then sonicated.

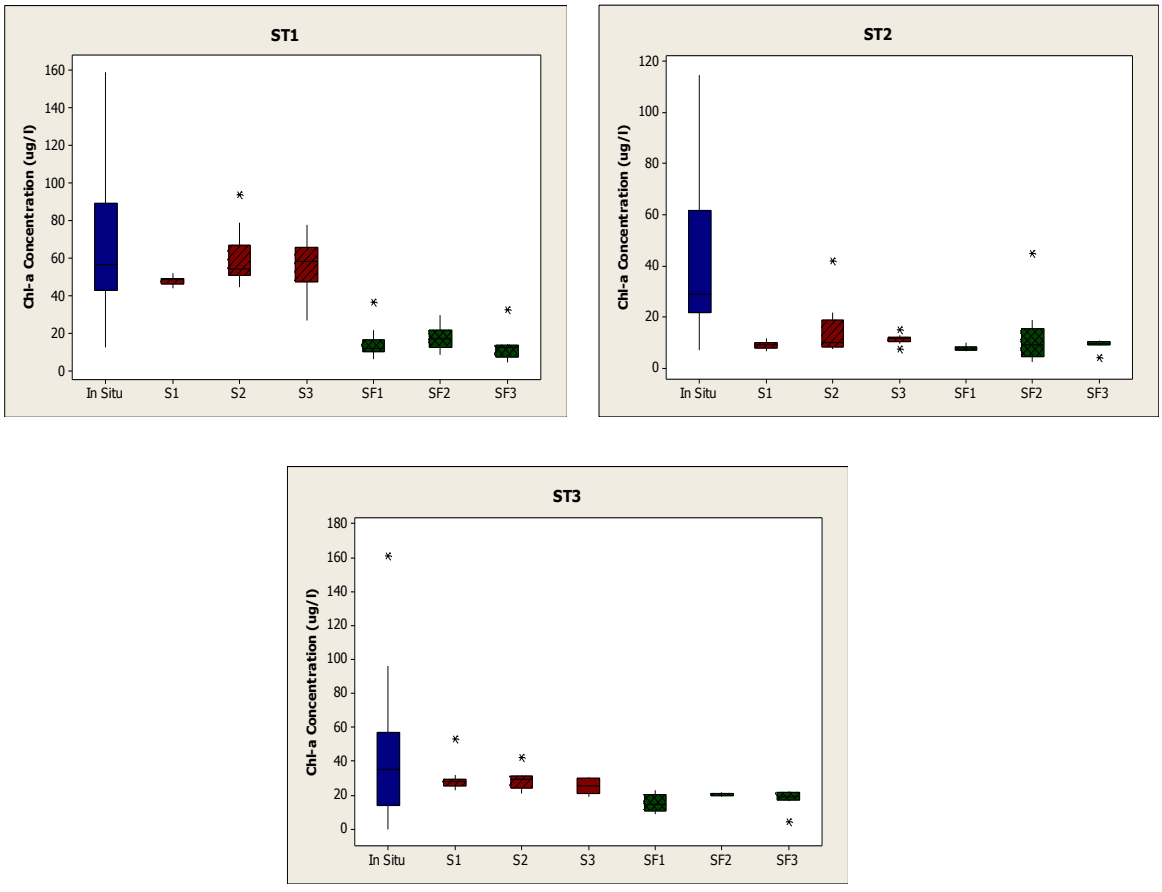


Figure III.5 displays boxplots for each pond showing results from readings taken; in-situ, after sonication, and after filtration prior to sonication. The results highlight the substantial variability associated with direct in-situ measurements, with a range of between 10-100 μg L^{-1} not uncommon. Unsurprisingly the greatest standard error and deviation were associated with this method (see table III.6).

Sonication of samples showed a greatly reduced range and substantial reductions in standard error and deviation, as highlighted in table III.6. Overall chlorophyll-*a* concentrations were also reduced somewhat from values obtained by in-situ measurements, potentially indicating an overestimation of concentrations using this method. Results from the filtered samples show further decrease in standard deviation and standard error, across ponds ST1 and ST2, with similar decreases in overall chlorophyll-*a* concentration also observed across all 3 ponds.

Reduction in mean chlorophyll-*a* concentrations from the filtration method suggested that chlorophyll-*a* cells bound together or to suspended particulates may be lost during this process leading to an underestimation of chlorophyll-*a* concentrations. It is also possible that degradation could occur given the time consuming nature of filtering samples. Furthermore, only marginal gains were achieved in terms of the improvement to standard error and deviation from filtration. It was decided that sonication of samples in the field was the most suitable and reproducible method, with which to proceed.

Table III.6: Results from the chlorophyll-*a* probe sampling technique trial. Values in µg L^{-1}.

READING	ST1			ST2			ST3		
	MEAN	SE	SD	MEAN	SE	SD	MEAN	SE	SD
IN SITU (25 READINGS)	67.70	8.06	36.95	44.30	10.30	34.10	42.20	8.88	39.70
SONICATED									
S1	48.27	0.79	2.63	8.97	0.52	1.57	30.04	2.72	8.61
S2	60.24	4.81	15.21	14.55	3.37	10.67	28.89	1.94	6.15
S3	55.71	4.98	15.74	11.30	0.63	1.99	25.88	1.43	4.52
Mean	**54.74**	**3.53**	**11.19**	**11.61**	**1.51**	**4.74**	**28.27**	**2.03**	**6.43**
FILTERED AND SONICATED									
SF1	15.04	2.75	8.68	7.79	0.31	0.96	15.69	1.57	4.98
SF2	17.95	2.02	6.39	12.78	3.92	12.40	20.68	0.27	0.84
SF3	13.17	2.38	7.52	9.22	0.61	1.92	18.19	1.63	5.16
Mean	**15.39**	**2.38**	**7.53**	**9.93**	**1.61**	**5.10**	**18.19**	**1.16**	**3.66**

3.0 Microbial 16s rRNA Analysis

A total of 99 samples were taken for 16s ribosomal RNA (rRNA) analysis. Samples were collected from 9 mature ponds (Ch VI) and the newly constructed ST ponds (Ch V) located at the study site. Replicate cores were taken for the ponds studied in chapters IV and VI, using the same coring device used in previous chapters.

Samples from this component were taken from the sediment cores forming the basis of chapter VI. A total of 10 samples were taken down the core profile for each pond at 1 cm resolution. The 3 new ponds sampled were those from chapter V (ST1, ST2 and ST3), only 2 samples were taken from these cores given the much shorter core depth. A triplicate of samples was also taken for an individual sample, to assess the degree of variation within an individual sediment layer.

3.1 16S rRNA Sequencing

One of the single most important questions in microbial ecology is “who is there?” (Sanschagrin, 2014). Sequencing of 16s rRNA gene amplicons generated by domain level PCR reactions amplifying from isolated genomic DNA is considered a “gold standard” in answering this question (Sanschagarin, 2014). Past techniques for the analysis of microbes in environmental samples have included the use of geochemical biomarkers such as groups of Geo and Bio-Hopanoids (Zhu et al., 2011; Cooke et al., 2008; Talbot et al., 2007). Previous sequencing techniques have been limited to the number of sequences that can be produced and relied on inserting genetic fragments into cultivatable communities. The advancement of this area to include technologies such as 454 and Illumina High Throughput Sequencing analysis mean that millions of sequences of genetic fragments can be produced, transforming the amount of insightful information available to identify microbial communities from isolated DNA (Kozich et al., 2013).

16S rRNA sequencing has fast become one of the quickest and most informative amplicon (rRNA fragment) sequencing methods used to identify and compare bacterial communities present within environmental samples. It is becoming increasingly well established as a method for observing the phylogeny and taxonomy of samples from complex environments that were previously difficult or nigh on impossible to study (Sanschagarin, 2014; Kozich et al., 2013).

Amplicon sequencing can target one of several genomic regions containing functional genes. By far the most popular option is to use marker genes within genomic DNA, such as 16S rRNA, which contains a number of highly variable regions (i.e. V3, V4, and

V45), which can be sequenced to identify community profiles (Sanschagarin, 2014). Next generation sequencing facilitates massive parallelisation of sequencing reactions, but more importantly, clonal separation of templates without the need to insert gene fragments into a host, which was required under previous sequencing techniques. In this study the V4 region of the 16s rRNA gene was targeted.

DNA was extracted and isolated from sediment samples. rRNA is then initially amplified by polymerase chain reaction (PCR) using primers containing sequencing adapters and unique barcodes. Samples were then loaded on to a disposable chip containing small sample wells and inserted into the sequencing machine. In this study an Illumina Next Generation Sequencer was used. Sequences were retrieved in a fastq file format and subsequently filtered to remove poor quality reads.

Filtered and binned reads can then be used in publically available bioinformatic tools, the most commonly used being Mothur and QUIIME. Bioinformatics in this study was carried out in Mothur. These software packages combine sample reads to create sequences representing complete rRNA profiles. Further data curation was carried out at this stage removing misaligned and chimeric sequences. Once this has been carried out sequence reads can then be referenced to published genetic databases such as Green Genes (DeSantis, 2006) and Silva (Quast, 2013), this study utilised the Silva database. Reads were classified to user defined taxonomic levels based on phylogeny or operational taxonomic units (OTU's), down to genus level. OTU's offer one advantage of traditional phylogenic analysis as they account for observed differences within phylum levels, between samples. The final step assigned the number of reads identified for OTU's in each sample.

3.2 DNA Extraction and Isolation

DNA was extracted from sediment core samples (0.25 g) using the PowerSoil DNA isolation kit (Cambio Laboratories), following manufacturers protocols.

Samples were initially homogenised and lysed in a buffer solution, used to facilitate soil dispersion, dissolve humic acids and protect nucleic acid from degradation. Sample were then briefly vortexed. SDS and other disruption agents required were then added for the requirements of complete cell lysis. SDS is also an anionic detergent that associates with and disrupts fatty acids and lipids associated with the cell membrane of various organisms. Samples were vortexed at maximum speed for 10 minutes to completely homogonise and lyse microbial cells, prior to centrifugation at 10,000 x g for 30 seconds.

Supernatant was transferred to a clean collection tube, where 250 µL of proprietary protein precipitation solution was added. The solution precipitates non-DNA organic and inorganic material including humic substances, cell debris and proteins that can reduce DNA purity and inhibit the PCR reaction. The solution was vortexed briefly and incubated at 5◦C for 5 minutes prior to centrifugation for 1 minute at 10,000 x g. This process is then repeated using 200 µL proprietary aqueous inhibitor removal solutions to remove additional non-DNA organic and inorganic substances.

1.2 mL of proprietary aqueous bind solution was added to the supernatant and vortexed briefly. This is a high concentration salt solution that facilitates the binding of DNA to spin filter whilst allowing other organic and inorganic materials to pass through. 675 µL of the sample solution was transferred to a spin filter and centrifuged at 10,000 x g for 1 minute, this process is repeated a further two times for the remaining sample. In this process DNA is selectively bound to the silica filter membrane in the spin filter whilst contaminants are able to pass through.

500 µL of an ethanol based wash solution was added to the spin filter and centrifuged at 10,000 x g for 30 seconds. This cleans DNA bound to the silica filter membrane removing residual salt, humic acid and other contaminants. Flow through was discarded and sample centrifuged again at 10,000 x g to remove residual ethanol wash solution, which can interfere with downstream applications such as PCR and gel electrophoresis.

The spin filter was then placed in a clean collection tube and 100 µL of elution buffer added to the centre of the white filter membrane and centrifuged at 10,000 x g for 30 seconds. The elution buffer passes through the membrane releasing DNA that was bound in the presence of the high salt solution.

3.3 Quality Analysis

3.3.1 Nanodrop spectrophotometer

DNA concentrations, 260nm/280nm and 260nm/230nm ratios were determined spectrophotometrically using a Nanodrop 1000 instrument. 260nm/280nm ratios represent contamination of DNA samples with humic substances whilst 260nm/230nm ratios represent contamination with protein based materials. Contamination with either two substances can inhibit the PCR reaction resulting in a substantially reduced sequence yield and poor results. Establishing 260nm/280nm and 260nm/230nm ratios is crucial to ensure the quality of data produced from the sequencing process.

Samples (n = 8) were selected to cover a range of different ponds, depths and included two of the triplicate samples. There was a general decrease in concentration in the lower core samples, which may be due the structural nature of clay and its impact upon the homogenisation and lysis steps. All ratios and concentrations fell within accepted guideline limits (Thermo Scientific T009-Technical Bulletin Nanodrop 1000 & 8000), so it was deemed that no further purification steps would be required.

3.3.2 Gel electrophoresis

Gel electrophoresis was used to assess the quality of extracted DNA. It operates on the principle of size and charge based separation, as DNA fragments hold a negative charge in the presence of ionic buffer solutions, due to the release of phosphate groups. Ionic buffer solutions used include Tris Borate Ethylenediamine tetra-acetic acid (EDTA) (TBE) or Tris Acetate EDTA (TAE). Agarose gel was used for fragment separation based on molecular weight. Poor quality DNA produces a smear when run, whilst high quality DNA produces tight bands of high molecular weight compounds within the gel.

3.4 Sequencing analysis

250bp paired end reads of the V4 region were amplified using the Schloss Lab universal primer set. Sequencing was carried out on an Illumina benchtop sequencing system and was subject to quality control procedures, based on the Schloss wet lab protocol, including; removal of ambiguous bases, filtering sequences for misalignment and removal of chimeric sequences.

Software analysis was carried out in Mothur. Fastq files from the sequence run were analysed in accordance with the Schloss Mi-Seq SOP (Standard Operating Procedure) published by Kozich et al (2013). In short the process involves:

1) Combining the forward and reverse reads for each sample
2) Reduce sequencing and PCR errors (including chimeric sequences)
3) Processing improved sequences (sequence counts and alignment to taxonomic reference files. In this study the SILVA reference database was used)
4) Sequence Clustering
5) OTU based analysis

After processing 5,160,505 sequences were obtained, which were subsequently classified into 99,889 OTU’s.

3.5 Control Samples

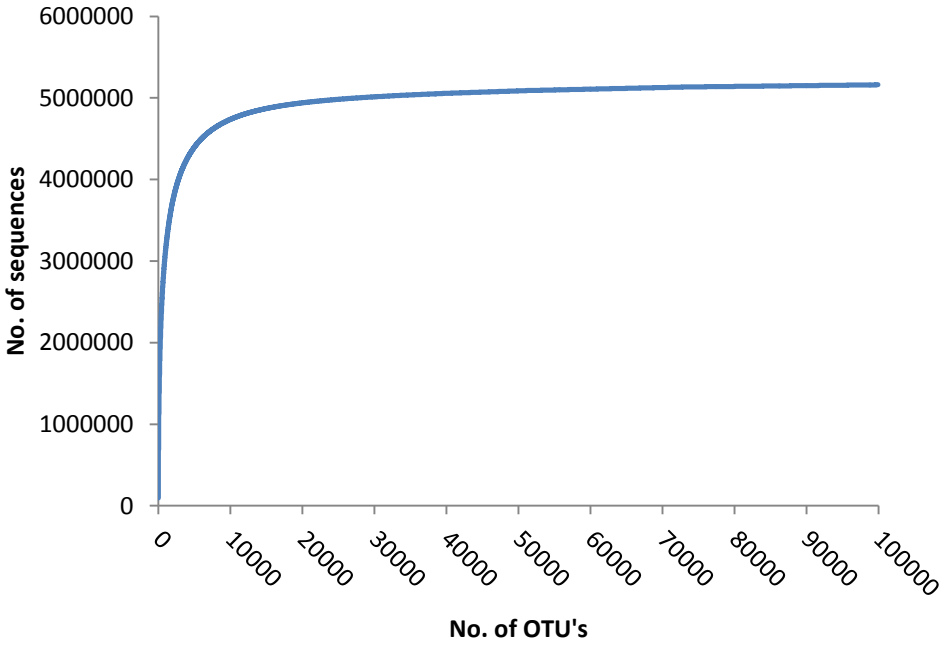
Positive controls (Mock community of *Halomonas* and *Streptococcus*) were used to ensure quality of the PCR reaction. Negative (deionised water) control samples are also included in the sample run to identify potentially contaminative OTU's. A total of 3 OTUs were deemed to be contaminative with a high number of sequences identified in the negative controls. These OTUs were removed from the analysis as were OTU's from the positive mock community prior to further analysis.

3.6 OTU Number and sequence reads

The number of phylotypes in each sediment sample was considered as the number of OTU's. Number of OTU's obtained depends on the phylogenetic distance considered. In this case a distance of 0.03 was used as outlined by Kozich et al. (2013).

Figure III.6 displays a rarefaction curve plotting number of OTU's against the total number of observed sequence reads. The curve shows that the majority of sequence reads are found across the first 10,000 OTU's with only marginal increases in the number of sequences obtained when further OTU's are taken. This graph confirms the use of the first 10,000 OTU's for beta diversity analysis.

Fig III.6: Rarefaction curve displaying number of OTU's and the number of sequence reads.



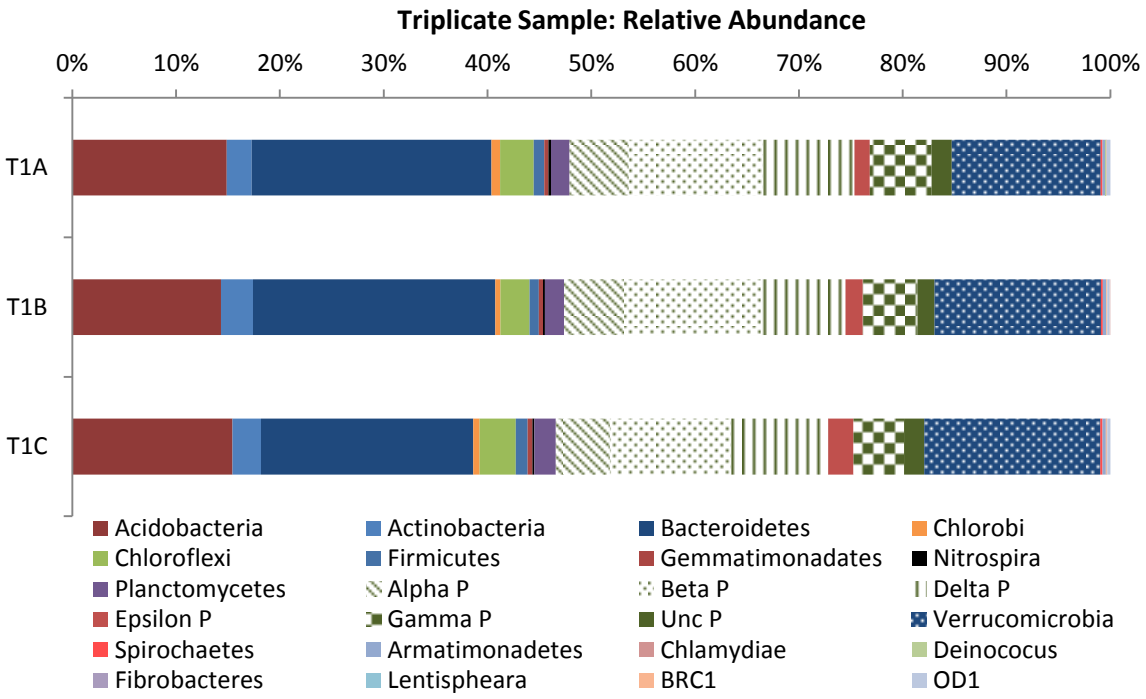
3.7 Sample Triplicate

A triplicate sample was included to determine whether microbial communities changed significantly within an individual sample (i.e. sediment layer). Relative abundance for each of the triplicate samples are shown in figure III.7. ANOSIM (Analysis of Similarity) was performed on the triplicate samples to identify any significant difference in community structure between the samples. No significant statistical difference was observed between the samples with all permutations yielding r-values of -1.00 and p-values of 0.5.

Results from this analysis will allow us to see that observed variations down the core profile and between individual ponds, will likely be attributed to actual changes in microbial community structure as opposed to within sediment layer variation.

This is important given the aims of this component are to assess broad changes in microbial community structure down the sediment profile and between ponds.

Fig III.7: Relative Abundance plots for the triplicate samples.



Chapter IV: Digging the Dirt on Carbon Storage in Constructed Ponds

Research Objectives:

1. Accurately quantify total OC storage and burial rates in three experimental ponds
2. Assess the percentage difference between sediment core estimates and whole pond OC storage
3. Compare OC storage and burial within the ponds to the surrounding soil

1.0 Introduction

Previous exploratory studies into OC storage within ponds have identified the following questions of importance:

> "How accurate are estimates of OC storage for a whole pond based on sediment cores?"

and

> "How does OC storage and burial in the pond sediments compare to surrounding terrestrial soils"

It is these questions upon which this chapter is primarily focused, trialling novel approaches of determining sediment-soil boundaries, whilst also obtaining quantitative values of OC storage and burial rates for a number of ponds at the Hauxley site. This chapter focuses specifically on the mature experimental ponds constructed in 1994.

Sediment cores in previous projects, focused on calculating OC storage in ponds across Druridge Bay, have been collected through a variety of methods including plastic tubing, dry ice corers (Gilbert et al., 2014) or Russian peat corers (undergraduate projects). Problems faced in the collection of anoxic sediments, usually less consolidated and higher in moisture content, coupled with substantial discrepancy between results of different coring methods, have led to major uncertainty in the effectiveness of such methods to yield reproducible results, allowing direct comparisons in sediment physicochemistry across different ponds (See chapter III, section 1.1) .

Sediment characteristics, chemical concentration and accumulation in inland waters are usually based on the collection of 5 - 10 sediment cores. Cores are generally spaced along latitudinal and longitudinal transects in order to account for patterns in sediment distribution (Pittman et al., 2013; Rippey et al., 2008; Shotbolt et al., 2006). However, there are limited studies that are able to quantify OC burial rates based on sediment volume, DBD and OC% values obtained from sediment cores. In large lakes, producing estimates generally involves using radioisotope dating or palynological methods to estimate rates of sediment deposition. Downing et al., (2008) used bathymetric surveys in agricultural impoundments over a number of years, to calculate water storage loss and net sediment accrual. This method is considered most accurate, as it does not require extrapolation from small area sediment deposition records. Pittman et al., (2013) is one of the latest studies to calculate OC burial rates from sediment cores, using sediment cores to estimate OC storage values and the known age of a recently constructed reservoir.

Furthermore, few studies have quantified the accuracy and precision of using sediment cores to estimate whole water body OC accumulation. Rippey et al. (2008) is one of the only studies to evaluate the accuracy of methods used to estimate whole-lake accumulation rates of OC. The study focussed on a large lake around 210,000 m^2 in size, and used 43 sediment cores along 8 transects to establish whole lake OC accumulation. The study found that using one central core to estimate whole lake OC accumulation resulted in an overestimation of OC by around 85%. Using OC concentration of the central site, alongside dry sediment accumulation rates in five other sites representative of sedimentation zones, reduced error to around ±10%. It was also reported that the use of 5-10 unrepresentative sites would still yield estimates with an associated error less than ±20%. Pittman et al. (2013) calculated the relative gains in precision achieved as a function of sampling effort. This study found that a minimum of 10 cores is needed to be representative of OC in small water bodies ranging from 50,000 - 250,000 $m^{2,}$ yielding a precision of ~25%. Drastic reduction in precision was observed using 5 cores (40% precision) and only marginal gains from taking more, with the use of 50 cores still yielding a precision >10%.

However, the Hauxley ponds are at least three orders of magnitude below this in area, so it is not necessary to take 10 cores from each pond. Although, due to the uniqueness of the exact known dimensions and distinct sediment-clay interface, ponds can be exhumed in their entirety to calculate exact values of total OC stored, a method not possible for larger water bodies. Therefore, an exact measure of inaccuracy associated with sediment core extrapolations can be produced, by comparing estimated OC storage values with total OC values from the pond exhumation. This will be the first study in this field to provide a baseline % difference in sediment core OC storage estimates to a total value. The newly developed corer (see chapter III) will facilitate the sampling of a range of ponds, producing accurate and reproducible results that will allow direct comparison between sediment cores.

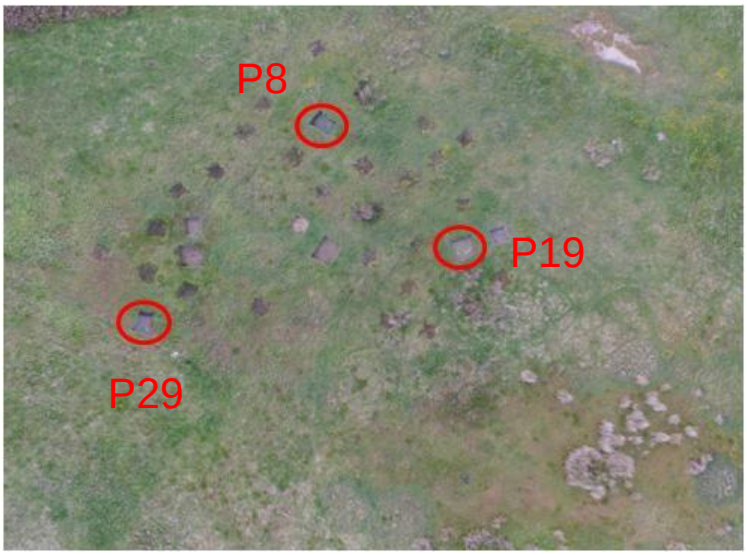
The distinct sediment-clay interface observed across the Hauxley ponds also allows a novel method of determining the boundary layer, between accumulated sediment and original bottom soil, in recently accumulated sediments to be trialled. A novel approach of exhuming the pond in its entirety was carried out, to accurately quantify OC storage and evaluate the % difference of sediment core estimates. Finally, the known construction date of the ponds and remediation of the site in this study facilitates the estimation of OC burial rates from sediment OC storage estimates. It also allows direct comparison between contrasting aquatic and terrestrial environments, informing their potential construction as natural carbon mitigation tools.

2.0 Methods

2.1 Pond Exhumation

Three ponds were selected for exhumation. Ponds were selected to be representative of the hydrological gradient of the field and the distinct vegetation community succession patterns observed (Chapter II). Ponds 8, 19 and 29 were selected and their position within the field can be seen in figure IV.1. It should be noted that prior to the exhumation of each pond, three sediment cores (see figure (III.1) were taken to assess the accuracy in estimating whole pond OC storage from a single sediment core.

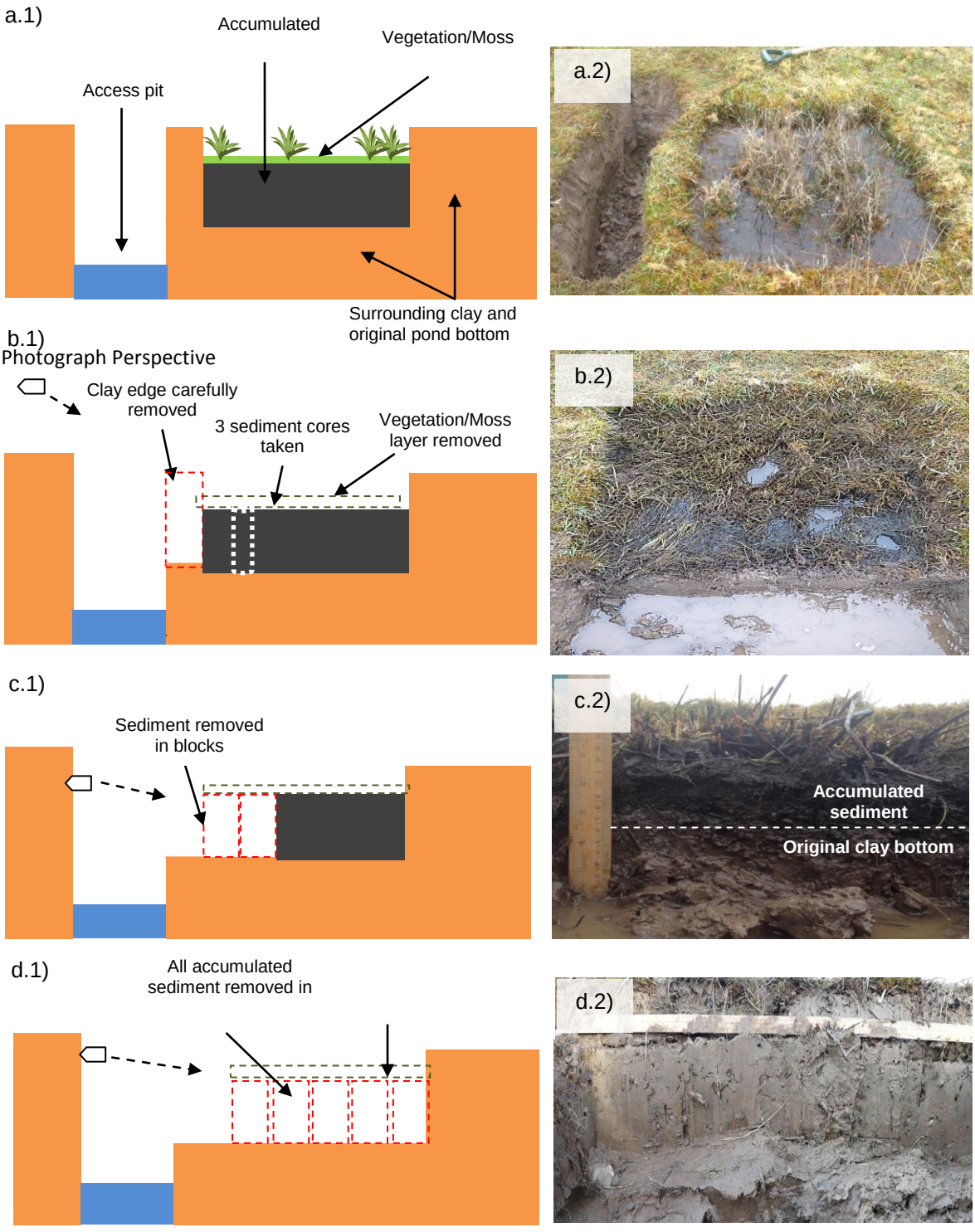
Figure IV.1: Site map highlighting ponds exhumed as part of this chapter.



Pond 19 was excavated in November 2012, pond 29 in March 2014 and pond 8 in April 2014.

Access to the sediment-clay interface was gained via the construction of an access pit at the edge of the pond (figure IV.2a). The pit was dug to be around 60 cm deep to allow surface water to drain out, whilst providing adequate depth to access the sediment-clay interface. Upon construction of the access pit, the soil edge was carefully removed, ensuring accumulated sediment was intact (figure IV.2b), to reveal the sediment clay interface (IV.2c). Surface layers of vegetation were removed first and stored in a cool box prior to transport back to the laboratory. Sediment was then removed in roughly 20 cm by 20 cm blocks, being careful to extract only accumulated sediment, till only the exposed clay sides remained figure IV.2c. All samples were wrapped in aluminium foil before transport to the labs, ensuring sediment blocks were kept upright at all times to avoid compaction of the sediment layer.

Figure IV.2: Conceptual and visual breakdown of pond exhumation methodology a.1) schematic cross section of access pit. a.2) Plan view of pond and access pit. b.1) Schematic cross section of cores taken and removal of final soil edge to reveal pond sediment. b.2) Plan view of sediment cores taken and the removal of soil edge. c.1). Conceptual schematic depicting the removal of sediment blocks once the sediment-clay interface can be seen. c.2) Photograph of the distinct sediment-clay interface. d.1) Conceptual schematic of removal of accumulated sediment. d.2) Photograph depicting complete removal of accumulated sediment.



Upon return to the laboratory, surface vegetation samples were weighed and placed in an oven to dry at around 60°C. Sediment block samples were visually inspected to remove any excess clay material that had not been removed in the field. Dimensions and wet weight of each sample were then calculated prior to air drying at 60°C for ~7 days. Samples were then reweighed prior to grinding and sieving. Two subsamples were taken from each sediment block sample to provide ~50 samples for EA (CN) analysis and small 5 cm x 5 cm blocks were taken prior to being air dried for DBD subsample analysis.

Total values of carbon were produced using the mass of the sediment block and mean OC% from two subsamples to estimate the mass of OC in each individual block. The mass of OC in all the blocks was then added together to produce a total mass of OC for the pond.

2.2 Sediment Cores

Taken prior to exhumation see section 2.1

The new corer, outlined in figure III.1, was used on Ponds 8 and 29. Plastic tube coring was used for pond 19 as this pond was exhumed prior to the new corer being developed, hence the lower resolution of sample points in comparison to the other two ponds.

2.3 Site Soil Comparison

Two soil comparison samples were taken from the surrounding soil immediately adjacent to ponds 8 and 29 upon their exhumation. Soil samples were taken in order to compare the surrounding soil profile to that observed within the pond sediment. These samples were extracted in a similar fashion to the removal of sediment blocks, and were subsequently dissected at ~1cm intervals, similar to sediment cores, in order to compare values to those observed in sediment cores.

3.0 Results

3.1 Exhumed Ponds

Basic descriptive data for the exhumed ponds can be seen in table IV.1. Sediment OC% ranged from 6.37 to 24.45 in all samples. Overall mean OC% between ponds was also fairly similar with values of 12.69% ± 3.86 observed in pond 8, 12.48% ± 3.83 in pond 19 and 12.68% ± 3.66 for pond 29. OC% displayed no significant statistical difference between ponds, table IV.2. However, a broad range was observed within individual ponds with values of 6.44 - 21.91%, 7.04 - 24.45% and 6.37 - 21.52% for ponds 8, 19 and 29 respectively see figure IV.3.

Table IV.1: Results from exhumed pond. * Adjusted to factor in the time difference between exhumations of ponds.

	Pond 8	**Pond 19**	**Pond 29**
Total Volume (cm^3)	49627.8	39650.1 (41852*)	45738.7
Total Sediment Dry Mass (g)	15330.5	13437.3 (14183.8*)	18631.2
Sediment Accumulation Rate (g yr^{-1})	766.50	746.52	931.56
Mean DBD (g cm^{-3})	0.30 ± 0.097	0.35 ± 0.129	0.49 ± 0.152
Range	0.14 – 0.59	0.21 – 0.77	0.27 – 0.89
Mean OC%	12.69 ± 3.86	12.48 ± 3.83	12.68 ± 3.66
Range	6.44 – 21.91	7.04 – 24.45	6.37 – 21.52
C Density (mg OC cm^{-3})	36 ± 9	37 ± 13	58 ± 10
Range	0.013 – 0.061	0.019 – 0.072	0.043 – 0.077
Total Carbon Stored (g)	**1729.13**	**1565.17 (1652.12*)**	**2288.77**
Burial Rate (g OC m^{-2} yr^{-1})	**91.01**	**86.95**	**120.46**

DBD appeared more variable between ponds with mean values of 0.30 ± 0.097, 0.35 ± 0.129, 0.49 ± 0.152 g cm^{-3} for ponds 8, 19 and 29 respectively. DBD also showed significant statistical difference between ponds (table IV.2). Ranges observed within individual ponds were variable with values of 0.14 – 0.59 g cm^{-3} observed in pond 8, 0.21 – 0.77 g cm^{-3} in pond 19 and 0.27 – 0.89 g cm^{-3} in pond 29 see figure IV.3. C density values observed across pond 8 and 19 were generally similar at around 036 ± 9 mg OC cm^{-3} and 37 ± 13 mg OC cm^{-3} respectively, whilst pond 29 displayed elevated levels, on average 58 ± 10 mg OC cm^{-3}. The range of values observed in individual ponds was equally variable (see figure IV.3). Statistical analysis of C density between the three ponds also displayed significant statistical difference (table IV.2).

Table IV.2: ANOVA results of 50 subsamples taken from exhumed pond sediment samples.

ANOVA Between Ponds	
Parameter	Sig
OC	0.859
DBD	<0.001
C density	<0.001

The overall mass of accumulated sediment dug out as blocks, varied across the ponds. The largest mass was observed in pond 29 followed by pond 8 and subsequently pond 19. Values obtained were 15330.5 g, 13437.3 g and 18631.2 g for ponds 8, 19 and 29 respectively. The lower values observed in pond 19 are likely a result of the earlier excavation of this pond in relation to the other two. To factor this out, sediment accumulation rates were produced by dividing overall mass values by the age of the pond at the time of excavation. We can see that adjusted accumulation rates are more comparable to those observed in pond 8, but remain substantially elevated in pond 29.

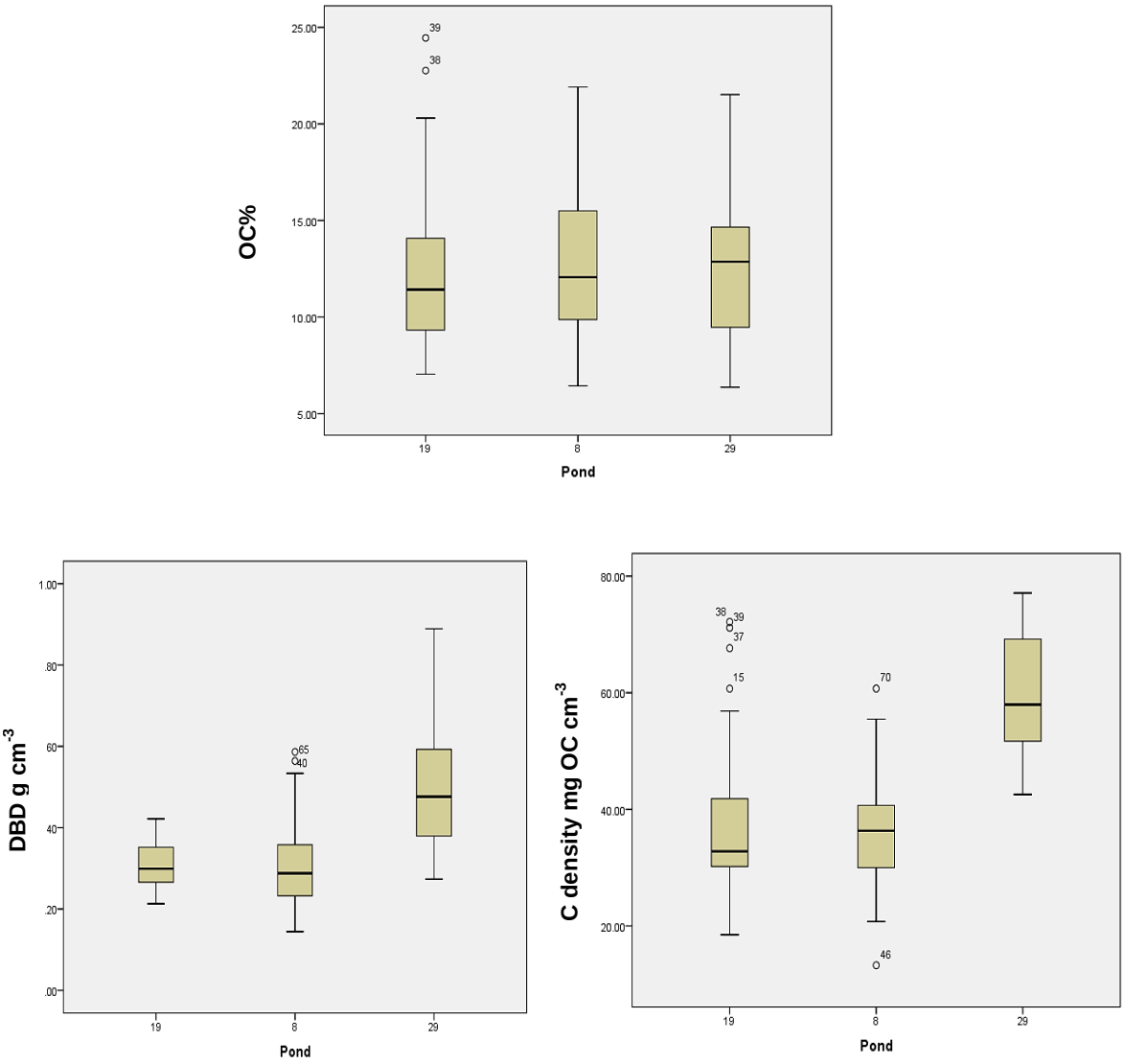
3.1.1 Sediment Cores

Sediment OC% ranged from 1.23 – 28.35% (n=96) but displayed substantial variation with core depth and between individual ponds (figure IV.4a). Highest OC% was found in upper sediment layers (to a maximum depth of ~8 cm) whilst OC% for the lower core layers beyond this were markedly lower.

DBD varied between ponds and with core depth (figure IV.4b). Upper core layers were generally characterised by lower DBD and higher variability between ponds, increasing with depth to a consistent level, which appeared uniform across all the three ponds, reflecting the transition from organic sediment into the clay bottom soil.

C density also varied with core depth and between ponds and was generally higher in the upper sediment layers before reaching a stable pattern with depth from around 3 cm (figure IV.4c).

Figure IV.3: Boxplots for results of 50 subsamples taken from exhumed pond samples.



3.2 Carbon Storage Estimates

Calculation: Total Carbon Stored - Exhumed Ponds

OC storage values were produced by using mean OC% calculated from ~50 pond samples taken from the sediment blocks, alongside the overall mass of accumulated sediment in order to estimate the mass of carbon accumulated within the sediment.

Mass of OC in individual blocks – Dry Weight/100 * mean OC% of two subsamples

Total Carbon Storage = Sum of OC stored in all sediment blocks

3.3 Sediment-Clay Interface Determination

Results from the XRF analysis can be seen in figures IV.5 and IV.6. Key elements are shown to provide an idea of their distribution and concentration down the core profile. The results highlight that elements associated with clay soils (i.e. aluminium, silicon, magnesium, potassium and iron; Nayak & Singh, 2007) generally increase in concentration with core depth to fairly stable concentrations in lower layers**,** whilst elements associated with organic sediment (i.e. sulphur and phosphorus) follow similar patterns to OC% decreasing considerably with core depth. Results demonstrate that these variables reflect chemical and physical changes down the core profile, particularly differences between accumulated sediment and original clay bottom soil.

A crucial component in estimating OC storage values from sediment cores to a whole pond, is the determination of the sediment and original bottom soil interface. The ponds at Hauxley were constructed in clay soil creating distinct chemical and physical differences between the accumulated sediment and original soil layers.

Determination of this interface is possible from a number of individual variables particularly OC% and DBD, as clay layers are noticeably denser and less rich in OC than accumulated sediment. However, discrepancy between variables across individual layers was apparent within sediment cores (figure IV.4), resulting in a degree of uncertainty in ascertaining the sediment depth layer required to accurately estimate whole pond OC storage values.

The observed results justify their use in multivariate analysis. Alongside other variables such as OC%, nitrogen and DBD, the sediment-clay interface can be comprehensively determined and used to accurately quantify OC storage and burial.

Figure IV.4: OC%, DBD, and Carbon density results for exhumed pond cores.

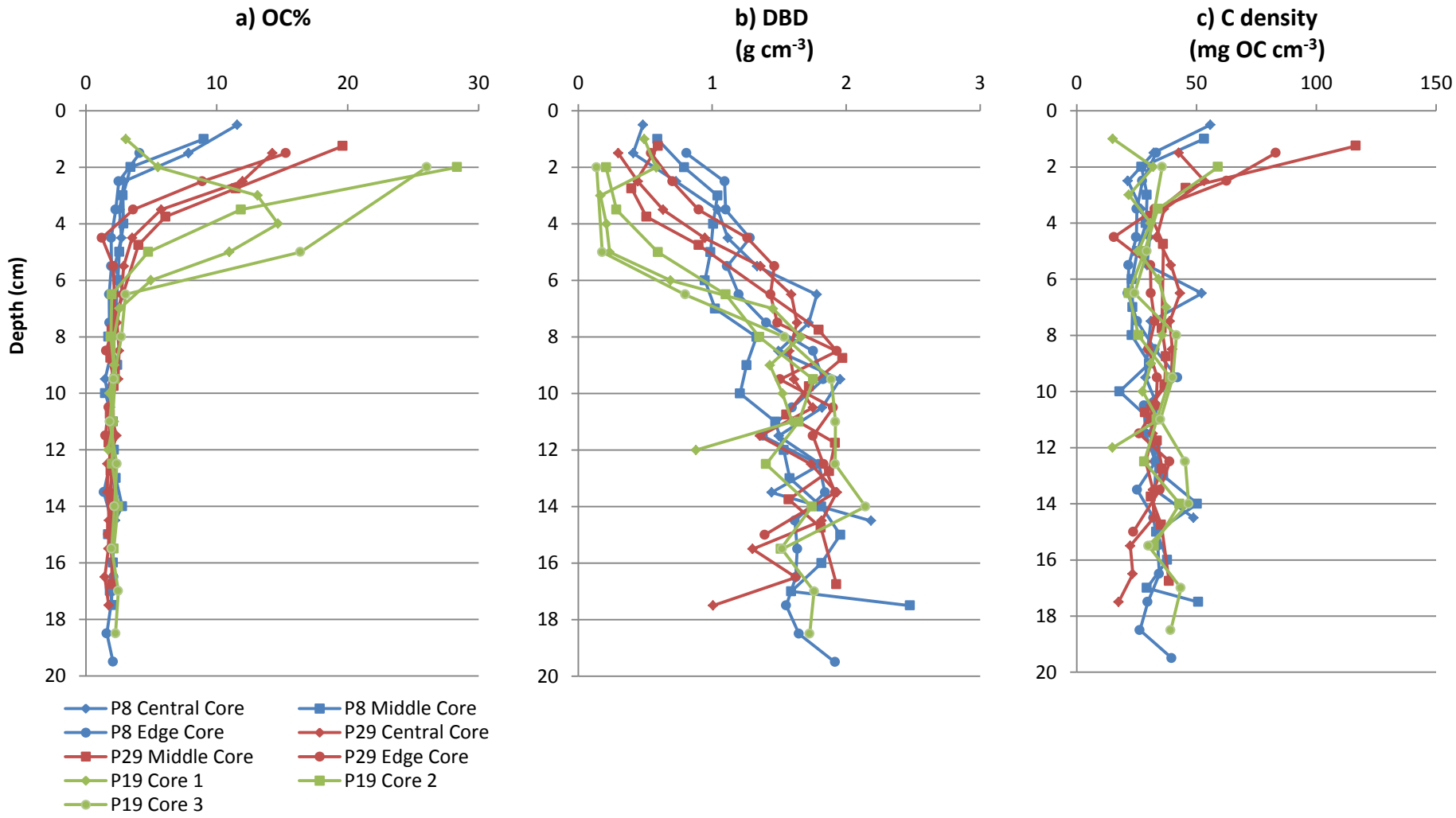


Figure IV.5: XRF results from exhumed pond cores (%N from TEA).

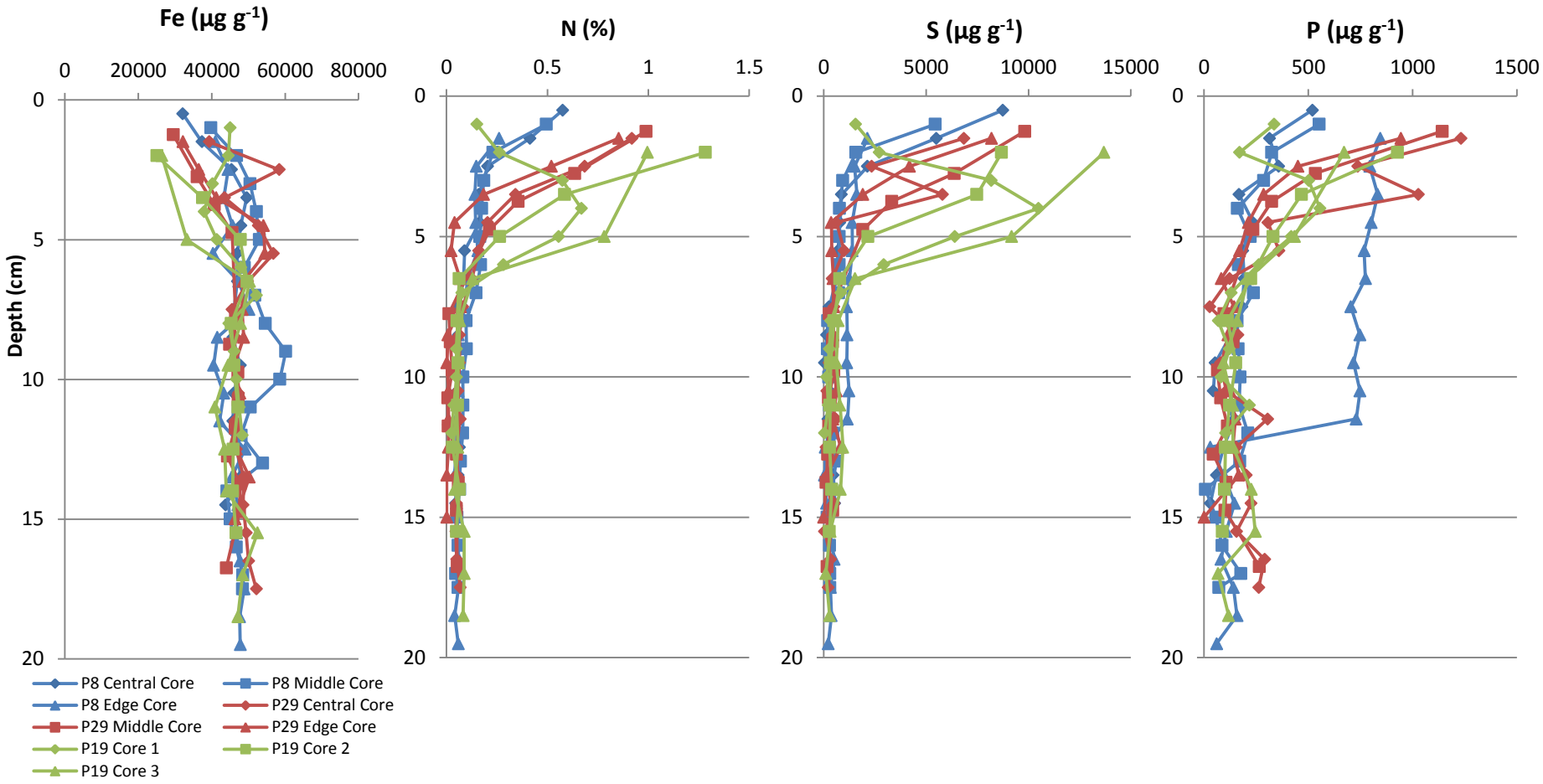
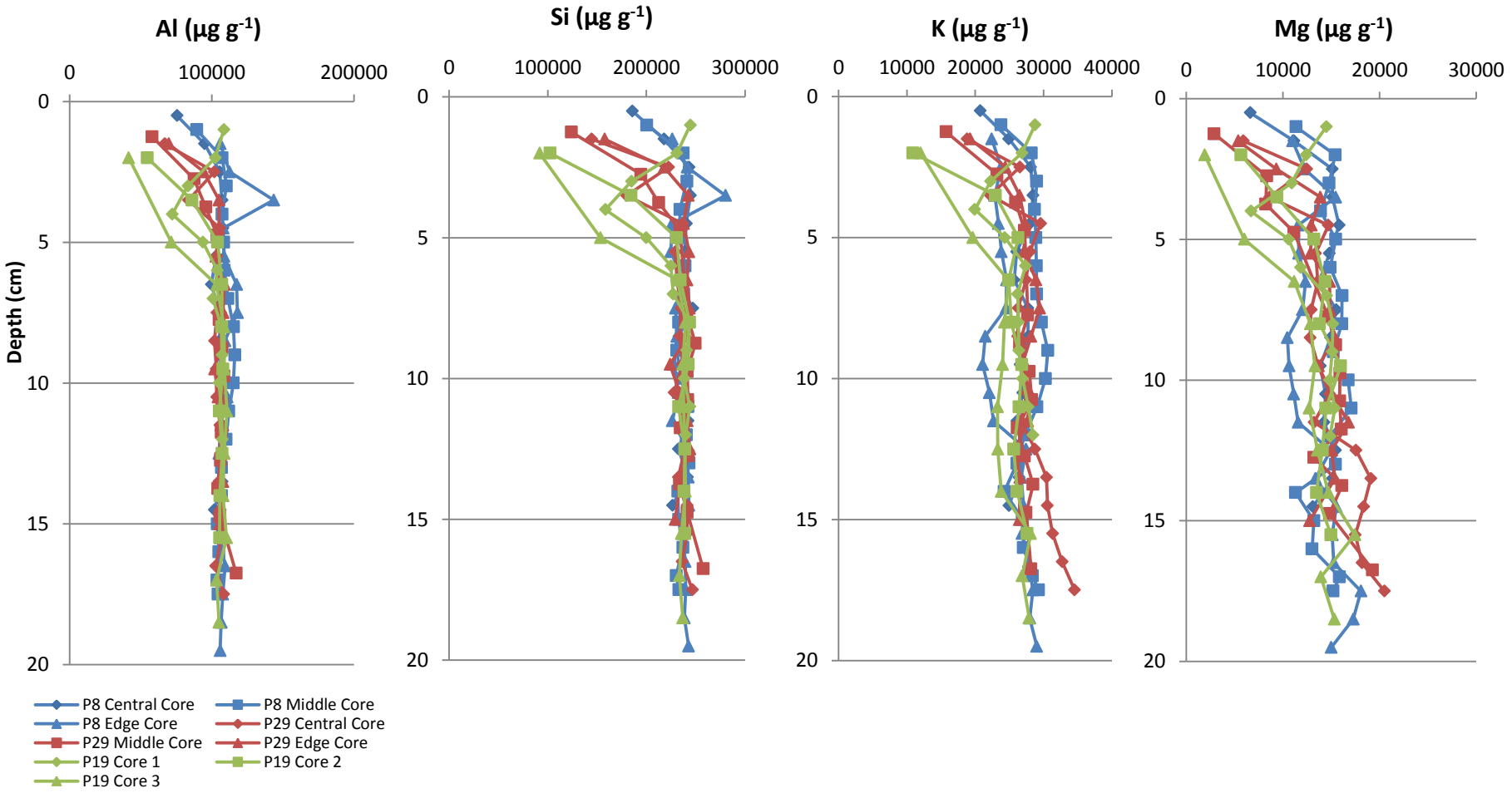


Figure IV.6: XRF results from exhumed pond cores.



3.3.1 Multivariate Analysis

In order to comprehensively determine the depth of the sediment-clay interface, Detrended Correspondance Analysis (DCA) was carried out to incorporate physical and chemical variables associated with sediment and underlying soil layers. Variables used to separate the distinct layers were OC%, nitrogen, phosphorus and sulphur for sediment layers, with aluminium, silicon, potassium, magnesium and iron used to represent clay soil layers. DBD was also used to separate points based on the physical characteristics of the soil.

Given the large range of measurements associated with sediment core variables, data was log 10 transformed to improve normality distribution of variables and distribution of sample points. Percentage of cumulative variance in the data explained by the DCA analysis was high, ranging from 73.3% to 95.79% on the first axis, to 91.50% to 98.11% on the second axis. DCA plots for individual cores from each pond can be seen in figures IV.7, IV.8 and IV.9 and have been included to demonstrate output from the DCA analysis and the identification of sample points used to determine the sediment-clay interface. All multivariate analysis was performed in CANOCO 5.5.

Table IV.3 highlights OC storage estimates based on the determined sediment depth fractions for individual cores and OC storage estimates. Estimates of total OC storage from cores taken from all three ponds ranged from 1565.90 to 2817.62 g OC m^{-2}, displaying considerable variation between ponds and individual cores.

Figure IV.7: Pond 8 DCA Analysis. Red circles indicate sediment layers.

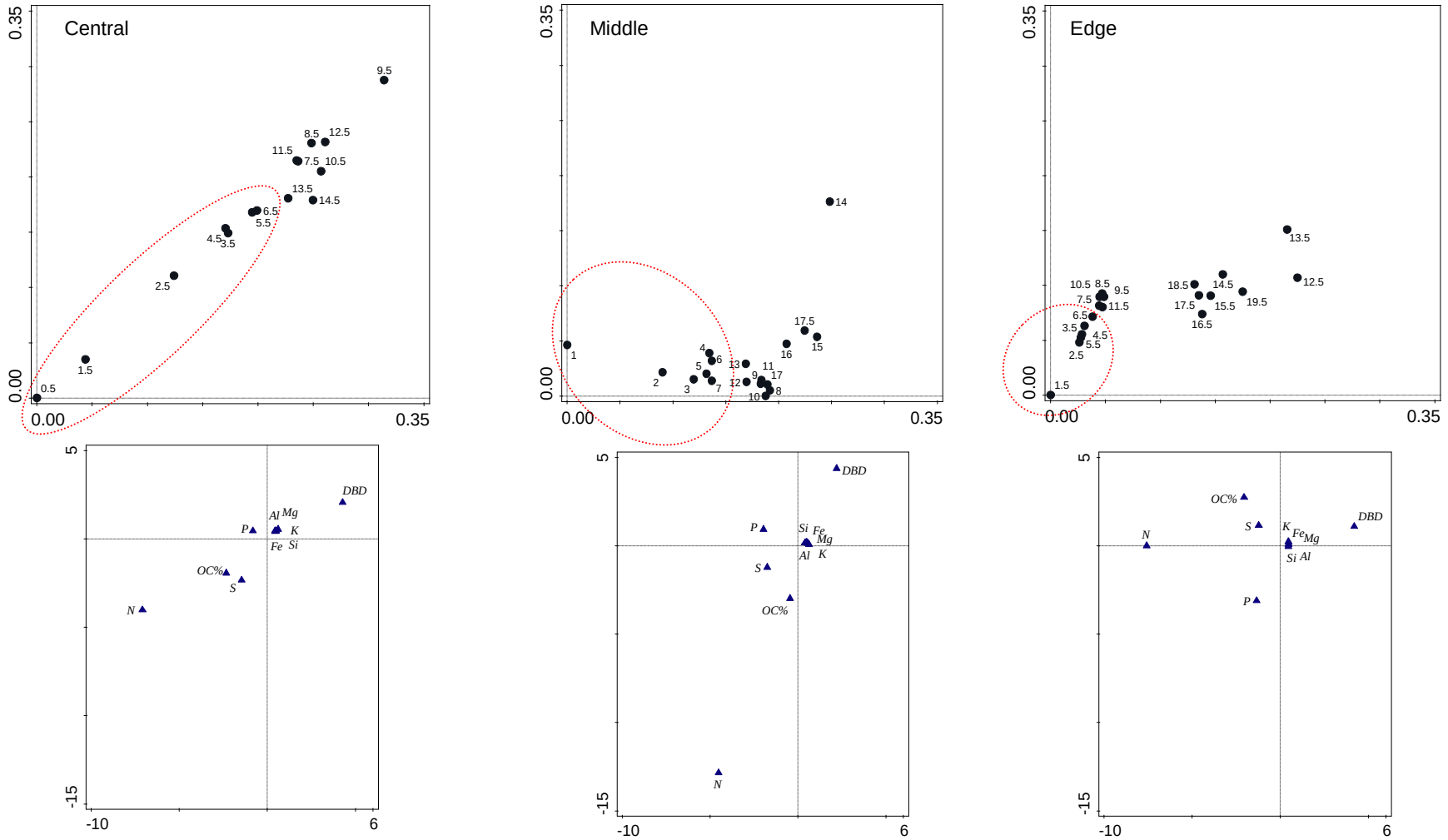


Figure IV.8: Pond 29 DCA Analysis. Red circles indicate sediment layers.

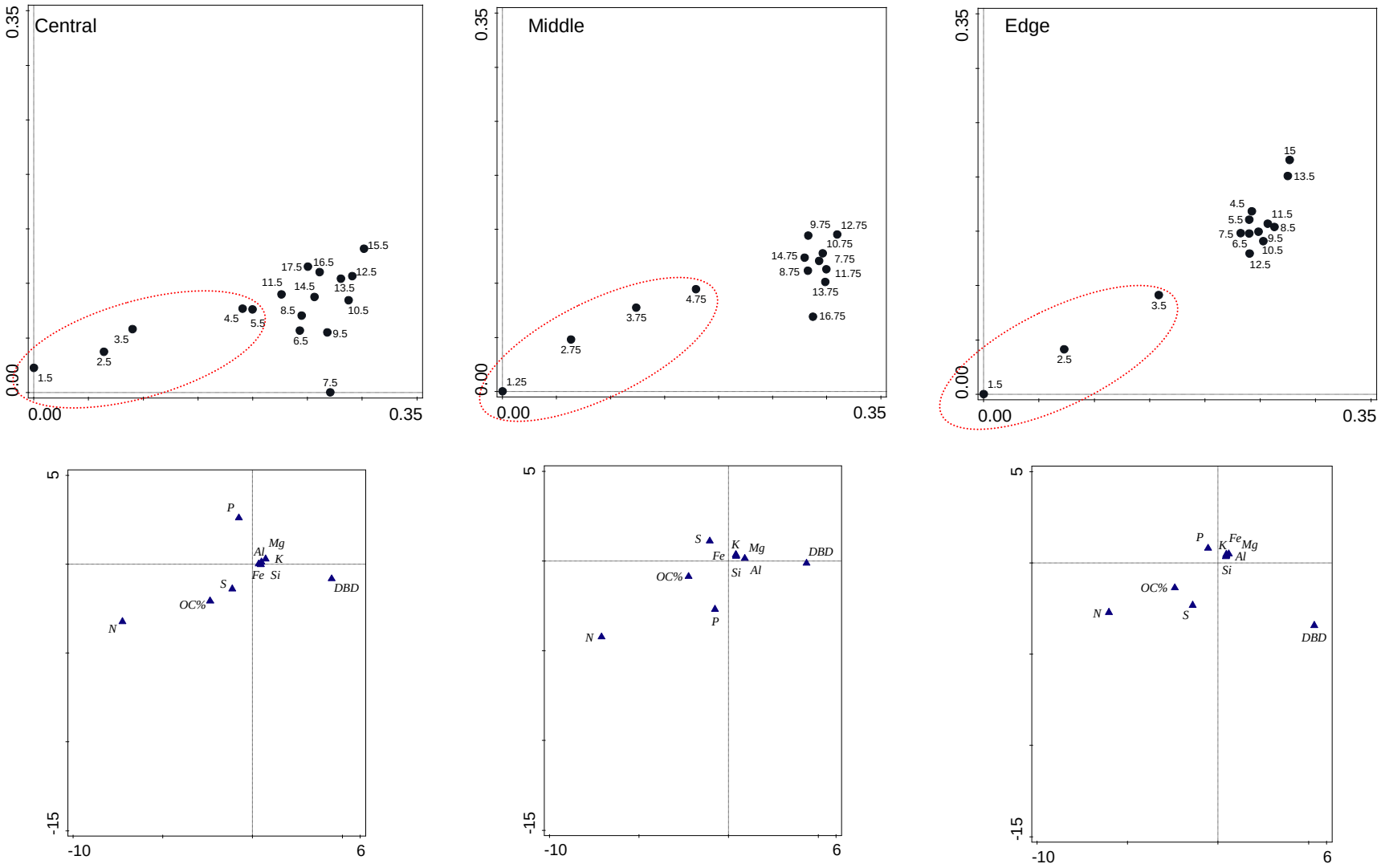


Figure IV.9: Pond 19 DCA Analysis. Red circles indicate sediment layers.

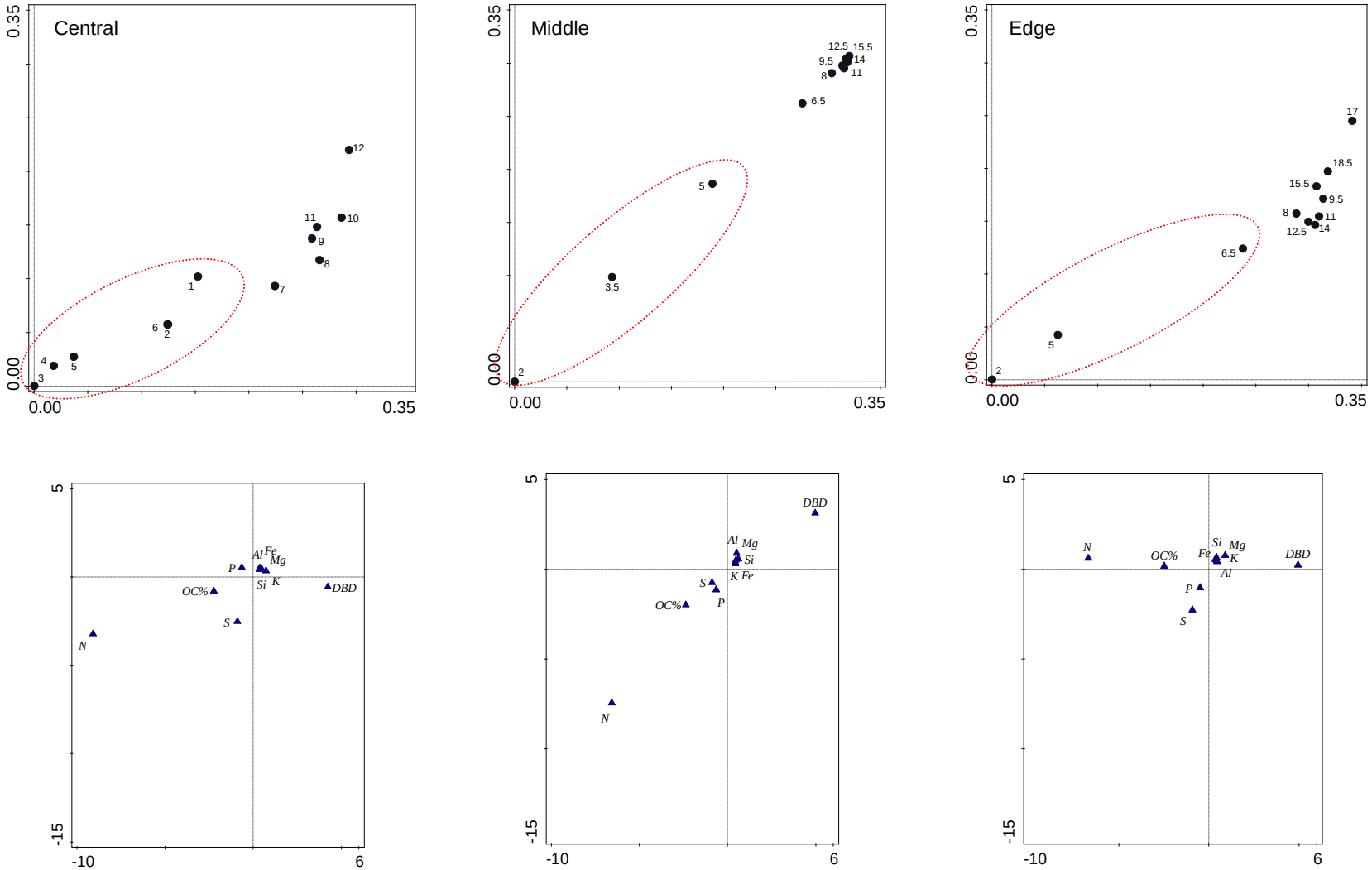


Table IV.3: Sediment-clay interface depth determined and associated OC storage estimate from the DCA analysis.

CoreID	Depth Layer determined from DCA Analysis (cm)	Carbon Storage Estimate (g)
P8 Central	6.5	2202.31
P8 Middle	7	2101.48
P8 Edge	6.5	2199.36
P29 Central	5.5	2263.22
P29 Middle	4.75	2817.62
P29 Edge	3.5	1702.05
P19 Core 1	6	1594.90
P19 Core 2	5	2112.08
P19 Core 3	6.5	1956.37

3.4 Assessment of Core Accuracy

OC storage estimates were produced for each sediment core based on results of the DCA analysis and subsequent extrapolation to provide an estimate of OC storage for the whole pond. Table IV.4 highlights the percentage difference between estimated OC storage values and those obtained from the exhumation of the whole pond. Percentage difference across individual cores ranged from a low of 1.12%, to a maximum of 34.94%, displaying an overall average difference of 12.33%. Estimates based on the mean of three sediment cores were more accurate, ranging from 2.65% to 12.57% with an average 14.14 % difference.

Table IV.4: Sediment core OC storage estimates and % difference between exhumed pond values.

Pond ID	Central core	Middle core	Edge core		3 Core Mean		Exhumed Pond Value
Pond 8							
OC storage estimate	2202.31	2101.48	1702.05		2001.95		1729.13
% difference to exhumed	+27.37	+21.53	1.57		+15.78		
Pond 29							
OC storage estimate	2263.22	2817.62	2199.36		2426.73		2288.77
% difference to exhumed	-1.12	+23.10	3.90		+6.02		
Pond 19							
OC storage estimate	1594.90	2112.08	1956.37		1887.78		1565.17
% difference to exhumed	1.89	+34.94	-24.99		+20.61		
Mean							
% difference		**Single Cores**			**3 Core Mean**		
		±15.60%			**+14.14%**		

Table IV.5 highlights the percentage difference of sediment core OC storage estimates obtained from the newly developed corer, omitting results obtained from the coring methods used for pond 19. Overall mean percentage difference for individual cores was reduced considerably to 10.58 and to 2.58% when using a three core mean.

Table IV.5: Sediment core OC estimates taken with new corer and % difference with exhumed pond values.

New Corer	Central core	Middle core	Edge core		3 Core Mean		Exhumed Pond Value
Pond 8							
OC Storage Estimate	2202.31	2101.48	1702.05		2001.947		1729.13
% difference to exhumed	+27.37	+21.53	1.57		+15.78		
Pond 29							
Pond 29	2263.22	2817.62	2199.36		2426.733		2288.77
% difference to exhumed	-1.12	+23.10	3.90		+6.02		
Mean							
% Difference		**Single Cores**			**3 Core Mean**		
		±13.09%			**+10.90%**		

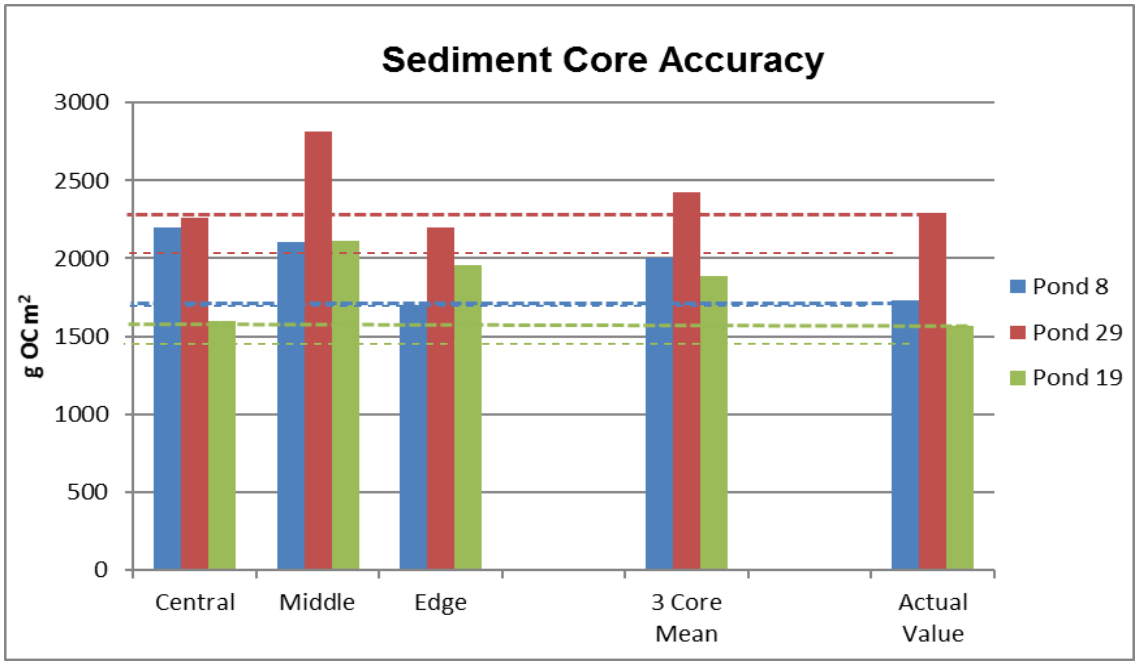
Table IV.6 displays the percentage difference values associated with pond 19 and the plastic tube coring method. Values for individual cores were considerably higher in comparison to those obtained from the new corer. The use of a three core mean using this method resulted in only a marginal improvement in overall accuracy and is perhaps attributed to the lower resolution of sub-sampling associated with using this method.

Figure IV.10 highlights estimated OC storage values for individual cores and an overall three core mean. Estimates were compared to values obtained from the whole pond exhumation. Results demonstrate the potential for sediment cores to both over and underestimate OC storage values, whilst highlighting a noticeable improvement in accuracy with the use of a three core mean.

Table IV.6: OC storage estimates for plastic tube cores from P19 and % difference to exhumed pond value

Pond 19	Central core	Middle core	Edge core		3 Core Mean		Actual Value
OC storage estimate	1594.90	2112.08	1956.37		1887.78		1676.96 ± 514.65
% difference to exhumed	1.89	+34.94	-24.99		+20.61		
Mean							
% difference to exhumed		**Single Cores**			**3 Core Mean**		
		±20.61%			**+20.61%**		

Figure IV.10: Graph showing sediment core accuracy for the three exhumed ponds.



3.5 Site Soil Comparison

OC% in soil comparison samples taken from cores adjacent to the exhumed ponds ranged from 1.59 – 16.48% (n=28) displaying substantial variation with core depth and to a lesser extent, individual soil samples (see figures IV.11 and IV.12). Highest OC% was found in upper soil layers (to a depth of 4 – 5 cm) whilst OC% the lower core layers below this showed marked decrease. Interestingly the pond 8 control sample showed elevated OC% above the sediment samples in the upper layer, whereas, in pond 29 the majority of sediment samples displayed elevated OC% in comparison to their respective soil comparison.

DBD ranged from 0.151 – 3.10 g cm^{-3} and increased with core depth (figures IV.11 and IV.12). Values were generally similar between soil samples and were similar to values observed across upper layers within pond sediments. However, a general elevation above sediment values was observed from around 5 cm downwards.

C density showed the most variability between the soil and sediment, displaying considerably lower values across the upper 2 cm and conversely higher values across 3-4 cm. Generally, values from 5 cm downwards were comparable to values observed within the sediments.

Figure IV.11: Pond 8 Soil comparison physicochemical results.

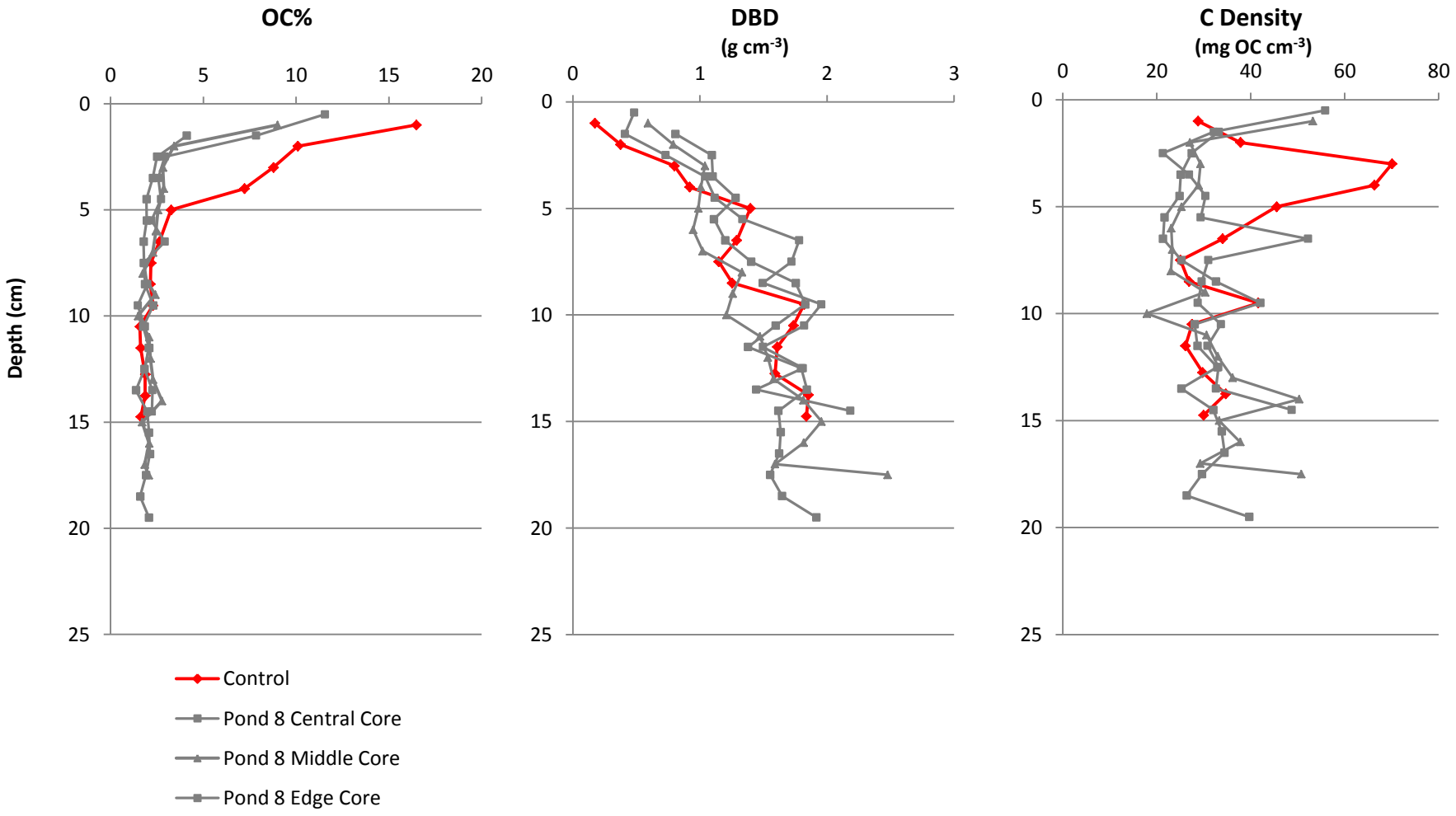
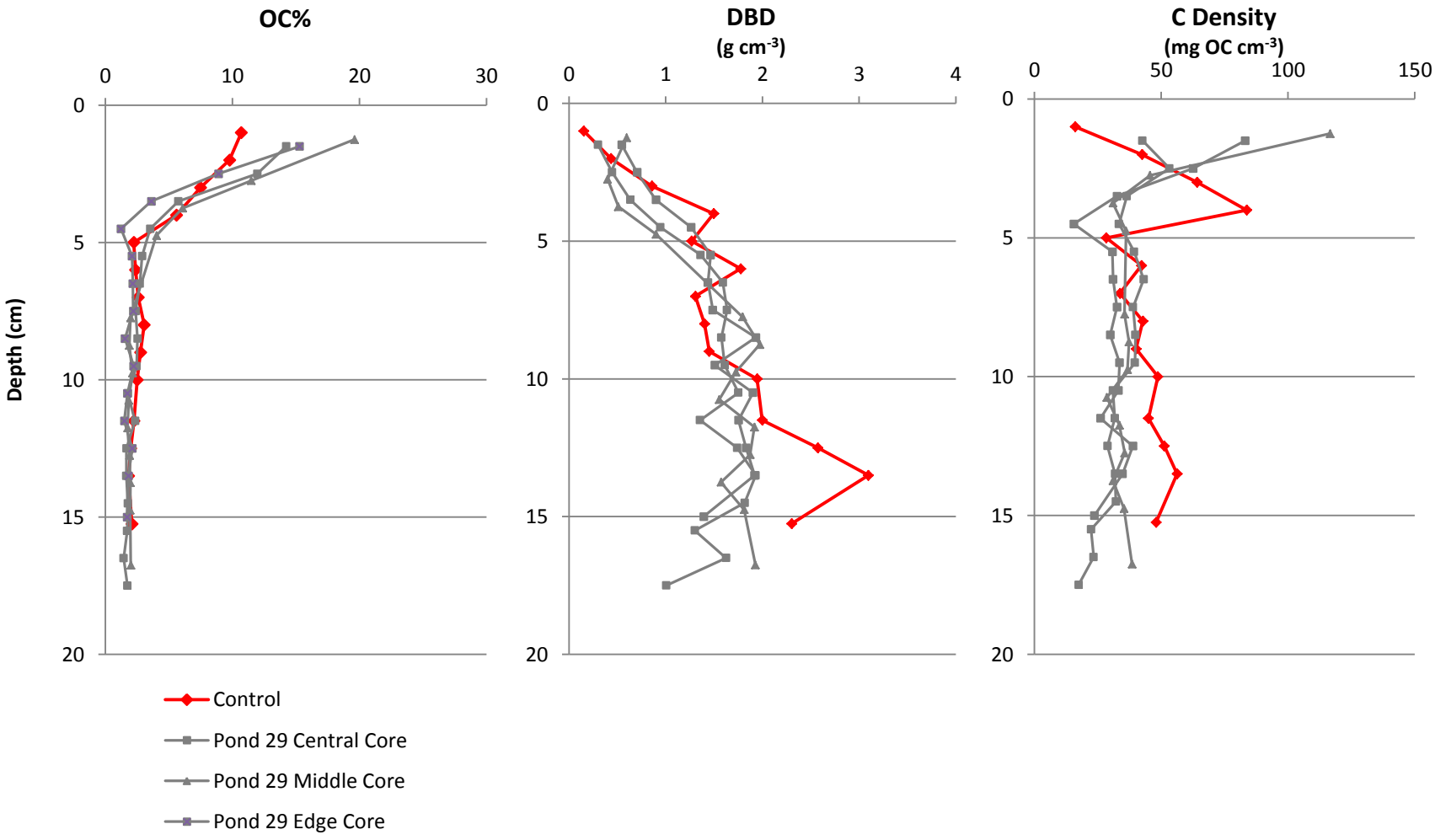


Figure IV.12: Pond 29 Soil comparison physicochemical results.



DCA analysis was conducted on the soil comparison cores to enable a similar fashion of determining OC storage in the surrounding soil (figure IV.13). Values of OC stored ranged from 2030.08 g OC m^{-2} in the pond 8 soil comparison, to 2066.14 g OC m^{-2} in the pond 29 soil comparison. Using the known age of the remediation of the site, burial rates have been estimated for top soil layers (table IV.7). However, it should be noted that these figures have been included purely as an exploratory comparison and may not be truly representative, as top soil was spread across the site upon its remediation.

Figure IV.13: DCA Analysis on soil comparison samples. Highlighted points indicate top soil layers.

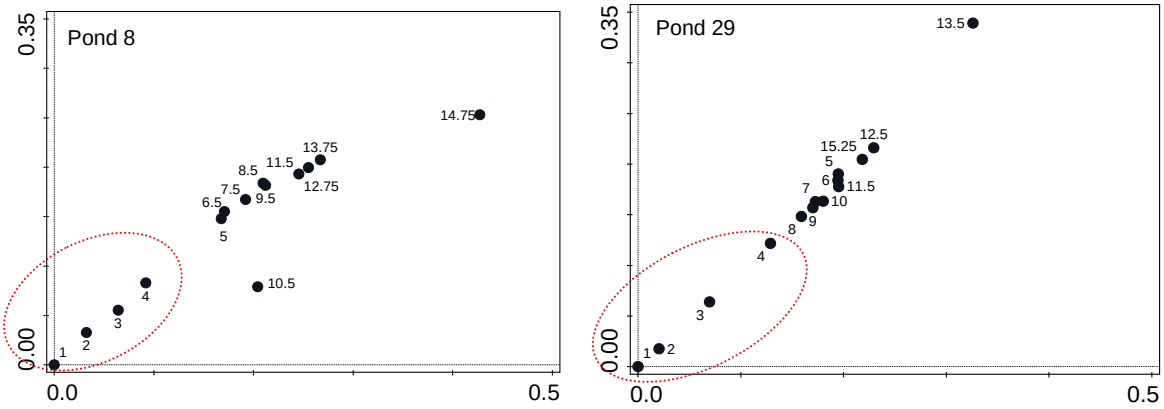


Table IV.7: Carbon storage and burial estimates for soil comparison samples.

	Depth Layer Determined	Carbon Stored in surrounding soil (g OC m^{-2})	Carbon Stored in pond sediment (g OC m^{-2})	Proposed burial rate for surrounding soil g OC m^{-2} (1974)	Burial Rate observed in Pond g OC m^{-2}
Pond 8	4	2030.08	1954.44	50.75	97.27
Pond 29	4	2066.14	2362.44	51.65	118.12

4.0 Discussion

4.1 Intra Pond Sediment Physicochemistry

Data collected from the exhumed ponds suggests a large degree of intra pond heterogeneity regarding physicochemical characteristics. Figure IV.3 highlights variation observed between 50 subsamples taken from each of the ponds for OC%, DBD and C density. The degree of intra pond variation is generally similar for the three physicochemical variables across all three ponds, although variability of DBD for pond 8 samples is limited to a much lesser extent that pond 19 and 29, indicating more similar sediment structures.

Factors potentially contributing to intra pond heterogeneity are likely complex and intrinsically related. Spatial patterns and coverage of both past and contemporary vegetation communities, driven by hydrology, are likely to be a predominant factor driving this spatial dissimilarity. However, this will no doubt be augmented by secondary factors, such as the presence and distribution of microbial communities involved in re-mineralisation of carbon. Other factors could also play a considerable role and include the relative proportion of inputs derived from allocthonous sources, known to be more resistant to degradation, bioturbation from benthic organisms or other fauna, and compaction of sediment due to trampling from larger fauna utilising the ponds.

Substantial intra pond spatial heterogeneity of physicochemical characteristics has potentially significant implications when attempting to sample these small ponds. Implications are largely revolved around the collection of sediment cores that are representative of the whole pond. Estimation of overall OC stored relies on the extrapolation of physicochemical structure and characteristics down the core profile, considerable intra pond heterogeneity could therefore result in gross over and under estimations of OC storage. The issue of deciding how many cores are needed to be taken to accurately represent and estimate whole pond OC storage should be carefully addressed.

4.2 Inter Pond Sediment Physicochemistry

Results from the three exhumed ponds suggest a degree of inter pond heterogeneity, particularly across DBD and C density see figure IV.3 and IV.4. Despite substantial intra pond variation in OC% observed across the three ponds, inter pond variation appears minimal with ponds exhibiting similar overall mean and range in OC%.

Statistical analysis of inter pond variation confirms that there is no significant statistical difference for OC% between the three ponds, despite significant statistical difference is observed for DBD and C density (see table IV.2). This appears to be driven largely by higher DBD observed in pond 29 in comparison to ponds 8 and 19, which were considerably lower, exhibiting a much smaller degree of variation from one another.

Increased DBD coupled with generally similar OC% values also result in a greater OC storage observed in pond 29 in comparison to pond 8 and 19. This suggests DBD and structural composition of the sediment has the potential to vary significantly between ponds and likely contributes to distinct variations in OC storage observed between the ponds. Dominant factors influencing DBD of sediment are likely associated with the development, growth and decay of vegetation communities within the pond. The establishment of root networks of certain vegetation communities, coupled with variability in the resistance of OM from different species to degradation, will undoubtedly affect the formation of sediment and its overall structural composition.

Other potential factors include levels of anoxicity in the sediment, which can result in the formation of sediments with higher moisture content, almost fluid like in nature and of a dark colour with a high organic content (Munsiri et al., 1994). Therefore individual hydroperiod of these temporary ponds may also be a contributory factor. Hydrology was associated with the deterministic development of vegetation communities, particularly the establishment of *L.riparium* (Jeffries, 2008), which was noted for its physicochemical effect on the sediment, keeping some areas damp and anoxic throughout summer drought periods (Gilbert et al., 2014).

4.3 Carbon Storage Values

OC storage values were produced using high resolution analysis of sediment OC% and the mass of exhumed sediment blocks. This is a novel approach to quantify accurate values of OC storage in ponds, which generally rely on sediment cores to estimate whole system storage values.

The uniform shape and small size of the ponds in this study facilitated this unique exhumation approach, in which highly accurate values of OC storage were calculated and the accuracy of sediment core extrapolations assessed. High resolution sub sampling has highlighted intra and inter pond variation, in both physicochemical characteristics and overall OC storage, within the pond sediment.

Estimated OC storage for the three ponds varied considerably, ranging from 1565.17 g C m^{-2} in pond 19 to 2288.77g C m^{-2} in pond 29 (see table IV.1). It should be noted that

OC storage values for pond 19 have been adjusted, using a calculated OC burial rate, to account for time differences in the exhumation of the ponds and make values more comparable to those obtained for the other two ponds.

Mean OC% is similar across the three ponds ruling this out as a primary factor driving variations in overall OC storage. Variations in OC storage between the ponds appear to be largely attributed to the overall mass of sediment accumulated over the ponds lifespan, alongside DBD and overall structural composition of the sediment. Pond 29 has a considerably greater DBD than the other two ponds, despite the total volume of sediment being less than that of pond 8 (see table IV.1). Although DBD in pond 19 is greater than that of pond 8, the greater total volume and total mass of accumulated sediment, resulting in slightly higher values of OC storage.

Results suggest that OC storage within ponds is largely driven by rates of material accumulation and the structural composition of sediment as opposed to the general OC% observed within the pond sediment.

4.4 Sediment Cores

Sediment cores taken prior to the exhumation of the three ponds, display generally similar physicochemical patterns down the core profile. However, considerable variations were observed in values obtained from individual cores from the same pond. OC% is generally highest at the top of the core, decreasing considerably down the core profile to relatively consistent levels, indicating a transition into a relatively inorganic environment, such as the original clay bottom soil. Similar patterns to OC% were also observed for nitrogen, phosphorus and sulphur. DBD generally increases down the core profile to a depth of around 6 - 8 cm, from which it adheres to a consistent range, a result of the more homogenous nature of the clay soil. Concentrations of other variables such as aluminium and silicon and to a lesser degree magnesium, potassium and iron, are associated with clay soils and follow similar to patterns to DBD, increasing down the core profile.

4.5 Carbon Storage Estimates

In figures IV.7, IV.8 and IV.9 the interface between accumulated sediment and original clay soil can be identified. DCA analysis was used to provide a comprehensive determination of the sediment depth layer as opposed to using a single value. For instance, using OC% as an example (figure IV.4), it would appear that the sediment-clay interface occurs between 4 - 8 cm for the majority of the cores. Similar threshold points can also be viewed amongst other physicochemical parameters, particularly

DBD, aluminium, silicon, sulphur and phosphorus. However, threshold points are not always uniform amongst parameters, leading to uncertainty and variability in producing OC storage estimates from a single variable.

Detrended correspondence analysis (DCA) was performed using key variables (OC%, N, S, Si, Al, Mg, K, Fe) in order to identify more comprehensively, the sediment-clay interface. Analysis of the plots reveals that predominant axis separating the data points are DBD and to a lesser extent variables associated with clay (Al, Si, Mg, K, Fe) followed by OC%, N and S on the opposing axis. DCA plots follow similar trends across the majority of the cores, with samples generally aligning successively along the OC% and DBD axes. Samples in the uppermost levels of the core (1 - 5 cm) aligned along the OC% axis, and follow a fairly sequential pattern reflecting sediment depth. Samples from lower depth layers aligned along the DBD axis, displaying less variability from one another and did not necessarily follow a sequential pattern. In samples located at the deepest points in the core, a tighter clustering of points was often observed.

The sediment-clay interface was determined by analysing the distribution of points and choosing the sample point at which there is clear separation between further points along the DBD axis. Table IV.3 highlights the depth layer at which the sediment-clay interface was determined for each core. This ranges from 3.5 cm to 7.5 cm, but were on average around 6 cm in depth. Discrepancies between ponds and within an individual pond, can arise from a number of reasons, particularly a naturally uneven distribution of sediment within the pond, which can also be compounded by compaction of upper sediment layers during the core extrusion process. Resulting associated OC storage estimates can also be viewed in table IV.3. Estimates range from 1594.90 g to 2817.62 g for whole pond estimates, similar to the range observed for OC storage obtained from the exhumed ponds (1676.96 g – 2362.44 g).

4.6 Sediment Core Accuracy

Accuracy of sediment cores was determined using values obtained from the exhumed pond and determining the % difference between these and sediment core estimations. Results from the analysis of the sediment core accuracy can be viewed in table IV.4. Percentage difference varied considerably for sediment core estimates ranging from (1.12% to 34.94%). The greatest degree of percentage difference was associated with single sediment cores with a mean difference of 15.60%. The calculation of a three core mean, resulted in some improvement in accuracy (figure IV.10) and a reduction to on average 14.14% difference.

Table IV.5 shows data for cores collected with the newly developed sediment corer and omits cores from pond 19 that were taken with plastic tubes. Average percentage difference observed for OC storage estimates based on cores from the new corer were considerably reduced. Percentage difference for single cores ranged from 1.12% to 27.37%, displaying an average of 13.09%, as opposed to 15.60% with the inclusion of pond 19 cores. The use of three sediment cores taken with the new corer yields an average of 10.90 % difference in comparison to 14.14% including pond 19 cores.

The high levels of accuracy and low percentage difference observed in this study, likely reflect the ratio of sample size collected to overall pond size. As the ponds are around 1 m^2 in area, the collection of sediment cores (equating to an area of 0.0017 m^2 each), results in the sampling of of 0.17% of the total sediment area. Increasing the number of cores considerably increases ratio of sediment sampled to overall area, with three cores covering 0.51% of the overall pond area, which coincides with a much greater improvement in accuracy.

However, studies on larger water bodies have very low ratios of sampled area to overall water body area. Therefore, error values are expected to be much higher. Pittman et al. (2013), stated a minimum of 10 cores is needed to be representative of water bodies ranging from 50 – 250,000 m^2, such a sampling density would represent only 0.000034 – 0.0000068% of total water body area. Similar levels of coverage are observed in the Rippey et al. (2008) study, which reported a ±10% error in OC accumulation rates through the use of 5 cores. Both studies were limited in terms of quantifying levels of accuracy due to unknown total values, which has been compensated for with high resolution sampling. In the case of the Rippey et al. (2008) study, 43 cores were taken from a lake with an area of around 210,000 m^2. Still this sample coverage only represents 0.000035% of the total lake area. The exact amount of OC storage within this study has facilitated the production of accurate values for the percentage difference of sediment core extrapolations, providing a first true baseline of accuracy for sediment core extrapolations in small inland waters.

Given that the percentage difference associated with the new corer was ~15% and only marginal improvement was achieved through the inclusion of further cores, it was decided that a single core should be sufficient. This would allow the sampling of a larger number of ponds and thus capture more comprehensively, variations between pond groups, allowing the significance of factors such as vegetation community succession, to be assessed.

4.7 Site Soil Comparison

When assessing individual variables observed in soil comparison samples, such as OC%, DBD and C density, results don't appear too dissimilar to those observed for sediment cores. OC% in the upper layers displayed both elevated and lower values to OC% observed in sediment cores (figures IV.11 and IV.12). DBD exhibited a similar pattern showing elevated and lower values adjacent sediments. Interestingly when combining the two variables to calculate C density in mg C cm^{-3}, the upper 2 cm were lower than those observed within sediments, whilst in the 3 - 4 cm layer they were considerably higher (figures IV.11 and IV.12). This displayed a pattern quite dissimilar to that observed within the sediment profile, where a generally higher C density was observed in the uppermost layers transitioning to consistent levels down the rest of the core profile, even across the sediment-clay interface.

When extrapolating to OC stored over a m^2 area, results follow similar values to that observed in the exhumed ponds. This suggests that these systems are not substantial stores of OC in comparison to the surrounding soil, storing comparable levels of OC, but occupying a much smaller areal coverage in relation to adjacent soils (table IV.7). The key result here lies within OC burial rates observed between the two contrasting systems, aquatic and terrestrial. Burial rates for the ponds were on average 107.70 g C m^{-2} yr^{-1}, whilst proposed burial rates for the adjacent soil were on average 51.65 g C m^{-2} yr^{-1} (table IV.7). This suggests that ponds store more than double the amount of OC, than the surrounding land area in a given year. It should be noted that burial rates for the soil comparison samples are exploratory and conservative, using an age factor of 40 years from the sites remediation in 1974. These calculations fail to account for the spreading of topsoil upon the remediation of the site and so a substantial proportion of the soil OC storage and burial observed may have been imported from this activity, opposed to natural accumulation the same way in which the constructed ponds have.

Published burial rates of carbon in temperate grassland ecosystems have reported burial rates of carbon in the region of 65 - 70 g C m^{-2} yr^{-1} (Abberton et al., 2007). A more comprehensive study by Schlesinger (1997) suggests that values may in fact be much lower, with a rate of around 2.2 g C m^{-2} yr^{-1} observed in temperate grassland soils and up to 12 g C m^{-2} yr^{-1} in temperate forests. Considering this, ponds clearly demonstrate the capacity to bury OC at rates up to an order of magnitude more than the surrounding terrestrial area. Results demonstrate the potential significance of small constructed ponds as substantial sinks of carbon, which raises implications for global carbon cycling and supports the construction of these features as natural landscape carbon mitigation features.

5.0 Conclusion

The results have provided insightful information as to the accuracy of coring methods. Plastic tube cores, such as those used in the Gilbert et al. (2014) are associated with higher levels of error. Despite this, observed OC storage and burial rates within the exhumed ponds are not too dissimilar for values reported in Gilbert et al. 2014. The study is the first to produce accuracy values for sediment core extrapolations based on estimated values (i.e. core extrapolations) to an exact total value (i.e. pond exhumation). As such, these results have provided an initial baseline of % difference, which can be used when extrapolating sediment cores to cover an entire water bodies area. Although, these values may not represent those observed in larger inland waters, they can certainly inform sampling efforts for water bodies <100 m^2, which are both numerous and not too dissimilar from the Hauxley ponds.

Results have also provided a direct comparison between aquatic and terrestrial systems in the same location. The ponds were found to bury at least double the amount of OC than the surrounding soil in a given year, though it is possible this figure will be considerably more given the conservative nature of soil estimates. Not only does this confirm the importance of small ponds in the global carbon cycle, it also highlights their effectiveness in the storage and burial of OC. Hauxley is a remediated opencast coal mine site, and was essentially a blank canvas after being capped with clay and top soil. This study has highlighted that the construction of habitats within this canvas can substantially alter the OC burial dynamics of the site, with the construction of small ponds considerably enhancing overall OC burial. This highlights the potential for these systems to be constructed across landscapes and implemented as natural features to mitigate rising carbon emissions.

Key Findings:

1. Accurately quantify total OC storage and burial rates in three experimental ponds

- OC storage values for the three exhumed ponds varied considerably and ranged from 1565.17 g OC m^{-2} in pond 19, to 2288.77 g OC m^{-2} in pond 29 , with respective burial rates of 86.95 g OC m^{-2} yr^{-1} and 120.46 g OC m^{-2} yr^{-1}.

2. Assess the percentage difference between sediment core estimates to whole pond OC storage

- % difference varied considerably for sediment core estimates ranging from (1.12% to 34.94%). The greatest degree of difference was associated with OC storage values estimated from a single sediment core.

- The addition of a three core mean resulted in a slight improvement in and a reduction to on average 14.14% difference.

- Average % difference observed for OC storage estimates based on cores taken with the new corer were considerably reduced. Percentage difference for single cores ranged from 1.12% to 27.37%, displaying an average of 13.09% as opposed to 15.60% using plastic tube cores.

- Results have provided the first ever baseline value for the % difference between sediment core estimates and whole pond OC storage, for other studies focusing on OC storage in small water bodies.

3. Compare OC storage and burial within the ponds to the surrounding soil

- OC storage between pond sediments and the surrounding soil were relatively comparable when extrapolated over 1 m^2.

- The key result lies within OC burial rates observed between the two contrasting systems. Burial rates for ponds were considerably higher than those proposed for adjacent soil samples, which were on average 51.65 g OC m^{-2} yr^{-1}. The results are exploratory but it is considered that these values may be notably less.

- Results support the potential of these features to be used in landscape carbon mitigation projects and included in current policy mechanisms, such as CAP.

Chapter V

Ponds, Probes and Productivity

Research Objectives:

1) Identify dominant controls on primary productivity in the ponds
2) Assess variations in physicochemical functioning between ponds
3) Assess the impact of initial physicochemical development on early OC burial and storage

1.0 Introduction

The importance of small aquatic systems in the conservation of local and regional aquatic biodiversity has gained increasing awareness and appreciation over recent years (Céréghino et al, 2013; Boix et al, 2012; Oertli, 2009; Biggs et al., 2005; Williams et al., 2004). Studies exploring the capacity of these systems to provide sustainable solutions to a number of key issues, particularly water management and climate regulation, are also increasing (Céréghino et al., 2013; Boix et al., 2012). As a result, our understanding and recognition of valuable services provided by small water bodies, such as water quality improvement, flood management and carbon sequestration, continue to develop (Tournebize et al., 2015; Anderson, 2013; Moore & Hunt 2012; Tang et al., 2013; Downing, 2010).

The role of ponds in global biogeochemical processes has received increasing interest over recent years, particularly in carbon cycling and storage (Gilbert et al., 2014; Downing, 2010; Battin et al., 2009). It is recognised they constitute a major fraction of the aquatic-land interface (Verpoorter et al., 2014). Littoral zones are often nutrient rich systems that facilitate rapid biogeochemical cycling, resulting in high levels of productivity (Lawniczak, 2010), which likely accounts for the disproportionate intensity of processes, including OC burial, observed in small aquatic systems in comparison to larger water bodies (Downing, 2010).

Physicochemistry and the trophic state of the water column are significant factors driving OC burial in aquatic systems (Heathcote, 2015; Anderson, 2014; Heathcote & Downing, 2012; Downing, 2010). Nutrient enhanced primary productivity in the water column, facilitates higher inputs of autochthonous OM, which in combination with loadings of allocthonous OM, results in sediment conditions that promote elevated rates of OC burial (Anderson 2014; Sobek et al., 2009; Tranvik et al., 2009; Cole et al., 2007). Variations in OC% and OC burial between lakes, different pond types and across superficially similar aquatic systems, have been attributed to differences in surrounding land use, hydrological regime, vegetation community, physicochemistry and the intrinsic interactions between these variables and pond productivity (Gilbert et al., 2014; Anderson et al., 2013; Downing et al., 2008).

Studies on small water bodies, ponds in particular, often fail to take into account the natural vegetation succession of these systems and its potential influence on OC storage and burial. Specifically, little is known about physicochemical changes that

occur as ponds progress through various stages of succession, essentially transitioning from a feature supporting vegetation of open water colonists and bare substrate to one which has developed a substantial, often OC rich, sediment layer supporting emergent vegetation species. (Angélibert et al., 2004).

Although Dr Jeffries monitored macrophyte and invertebrate communities for 20 years, there is limited data on physicochemical and productivity dynamics for the Hauxley experimental ponds. Understanding this vital component influencing OC burial is pivotal to comprehensively understanding factors driving the enhanced OC burial observed in ponds. Utilising the detailed ecological site history (Jeffries, 2008), would indicate that ponds with higher OC% and OC storage had developed thick swards of moss (*Leptodicytum riparium*) and grasses (*Agrostis/Alopecurus*) earlier than other ponds, which had retained a more aquatic based flora of Stonewort (*Chara vulgaris*) or Water Buttercup (*Ranunculus aquatilis*) (Gilbert et al., 2014). Spatial variations in the development of plant communities were determined by pond hydrology, particularly patterns of wetting and drying, which were themselves attributed to a ponds specific position within the field (Jeffries, 2008). It was speculated that hydrological patterns created distinct physicochemical gradients between ponds, promoting earlier establishment of plant communities associated with higher OC storage and burial rates (Gilbert et al., 2014).

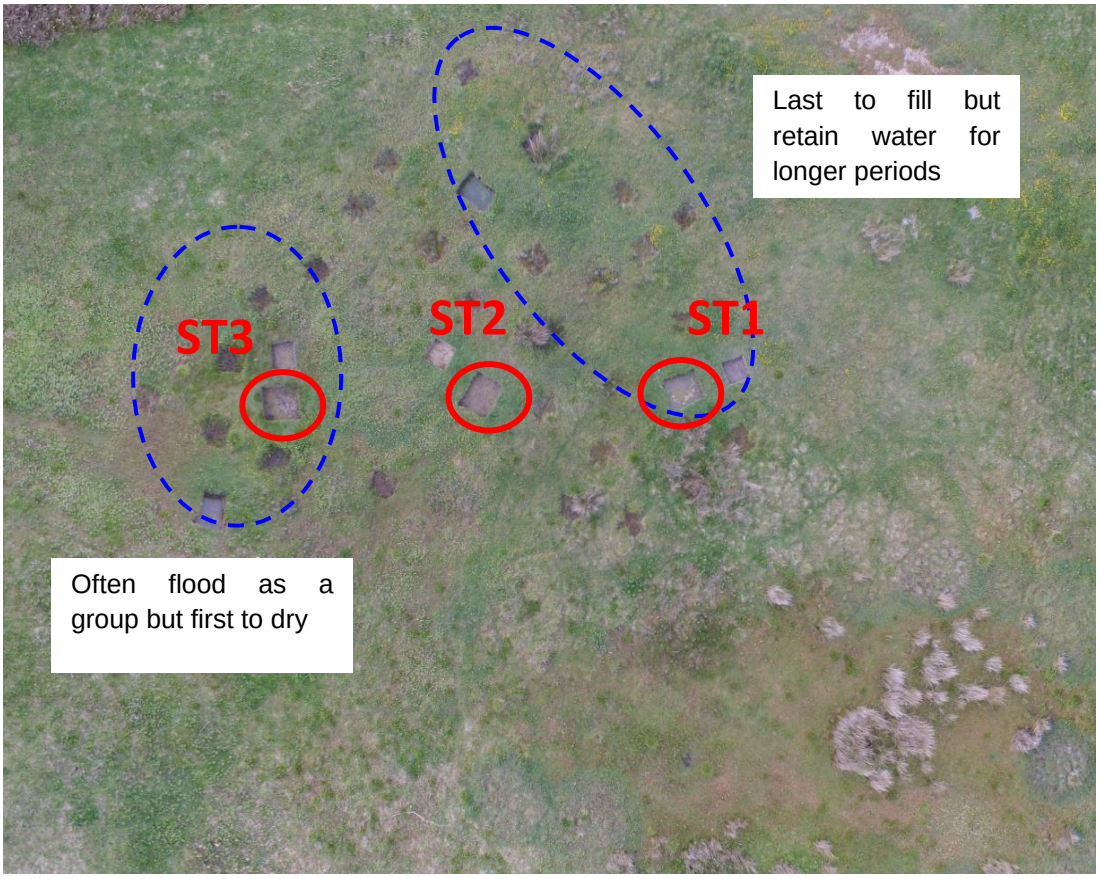
In this study, three new ponds were constructed in the experimental field site at Hauxley Nature Reserve, replicating those from previous studies on the heterogeneity of vegetation community succession, macroinvertebrate colonisation and sediment OC chemistry (Gilbert et al., 2014; Jeffries, 2010; 2008). The primary aims of this study were to monitor the evolution of physicochemical and productivity dynamics in the water column and its effect on sediment development after three years, identifying dominant controls on pond productivity, differences in physicochemical functioning between ponds and exploring the implication for OC storage and burial throughout early stages of pond succession.

2.0 Methods

2.1 Experimental Ponds

The ponds were constructed across the winter period 2012/13. The location of each individual pond can be seen in figure V.1. Ponds were constructed to be as close to replicate systems of the experimental ponds constructed in 1994. Ponds were sampled at approximately fortnightly intervals across the study period April 2013 – April 2016, offering high resolution insights into climate driven hydro-period and physicochemical development of the ponds over time, alongside spatial variations over the site.

Figure V.1: Satellite image of the Hauxley site highlighting the newly constructed experimental ponds.



2.2 Physicochemical Analysis

Analytical methods used for physicochemical methods are outlined in chapter III section 2.0. The development of a suitable and reproducible method for analysis with the chlorophyll-*a* probe is also outlined in chapter III.

2.3 Climate Data

Climate data was obtained from the Met Office, from the nearest station to the study site based at Boulmer Airfield roughly 15 miles north of the study site. Data obtained was mean weekly temperature and weekly rainfall. Climate data was transformed for later analysis using rolling averages for temperature, and cumulative values of rainfall. Both of which were calculated for 6, 5, 4, 3, 2 and 1 monthly time intervals and weekly time intervals of 6, 5, 4, 3, 2 and 1 weeks.

Stepwise regression analysis to identify climate variables that best described pond depth and ultimately hydro-period was performed in SPSS statistical software package.

2.4 Sediment Cores

Sediment cores were extracted using the newly developed stainless steel 4.7 cm diameter corer as outlined in chapter IV.

2.4.1 Elemental analysis

Analysis for OC and Nitrogen were analysed via elemental analysis. Samples were analysed on a Thermo Scientific Flash 2000 Organic Elemental Analyser configured to determine CN.

2.4.2 XRF

Samples were first placed in a ball mill for 180s before being made into pellets. Pellets made up of ~4 g of sample and ~0.7 g of FluXana CEREOX Licowax binder. Samples were then analysed via a Spectro X-lab 2000 and Spectro XEPOS.

3.0 Results

3.1 Summary Data

Summary statistics for depth and physicochemical variables analysed throughout the study are shown in Table V.1. Table V.1 illustrates mean values, standard deviation, maximum and minimum values observed over the study period. Results highlight both temporal and spatial variations in physicochemistry between the 3 ponds. Variations were particularly apparent in NO_3^--N and PO_4^{3-} chlorophyll-*a* concentrations between ponds, with ST1 displaying concentrations considerably higher than other ponds.

Table V.2 highlights mean values and standard deviation for individual ponds over the individual years of the sampling period. Results highlight inter pond heterogeneity as well as annual variability in the response of physicochemical variables.

3.2 Descriptive Data

3.2.1 Hydrology

Ponds followed similar hydrological patterns throughout the monitoring period, though both spatial and seasonal variations in dry-phases and overall water depth between the ponds were observed. Depths recorded for individual ponds across the sampling period plotted alongside monthly rainfall are displayed in figure V.2.

Temporal similarities in increasing water column depth and rewetting of ponds appear closely related to monthly rainfall. This is perhaps most apparent from December 2015 where sustained periods of heavy rainfall (Storm Desmond) resulted in extensive flooding of the study site, that sustained right until the end of the sampling period in April 2016. However, spatial variations were observed across hydro-period in individual ponds, particularly the susceptibility of ST2 and ST3 to dry-phases in drier periods.

3.2.2 Chlorophyll-a

Figure V.2 highlights chlorophyll-*a* concentrations observed for individual ponds across the sampling period. Productivity levels were high in all 3 ponds with chlorophyll-*a* concentrations displaying maximum levels of 276.66, 131.11 and 143.08 µg L^{-1} throughout the study period for ST1, ST2 and ST3 respectively. Overall mean concentrations varied between ponds with ST1 displaying on average higher chlorophyll-*a* concentrations (49.97 ± 47.79 µg L^{-1}) than ST2 (37.11 ± 29.90 µg L^{-1}) and ST3 (40.81 ± 32.35 µg L^{-1}) indicating a degree of spatial variation in productivity across the 3 ponds.

Table V.1: Basic descriptive physicochemical statistics observed across individual ponds throughout the study period. March 2014 – April 2016.

ST1	Mean	SE Mean	StDev	Min	Max
Depth (cm)	14.32	0.95	7.30	0.00	23.80
Conductivity (µS)	527.30	21.70	159.50	215.20	888.20
Temperature (°C)	13.64	0.82	5.98	2.87	23.39
Dissolved oxygen (mg L^{-1})	9.31	0.42	3.07	3.00	16.16
NO_3^--N (µg L^{-1})	5014.00	829.00	6093.00	133.00	28467.00
PO_4^{3-} (µg L^{-1})	688.00	109.00	799.00	117.00	4480.00
pH	7.53	0.092	0.67	6.37	9.48
Turbidity (FAU)	135.80	26.80	191.60	3.00	918.00
Chlorophyll-*a* (µg L^{-1})	49.97	6.50	47.79	0.00	276.66
Ammonia	72.00	15.00	49.00	27.00	183.00
Nitrite	8.00	3.00	11.00	0.00	37.00

ST2	Mean	SE Mean	StDev	Min	Max
Depth (cm)	11.34	1.17	8.91	0.00	23.55
Conductivity (µS)	561.00	20.30	138.00	360.50	938.00
Temperature (°C)	13.32	0.96	6.44	2.620	24.35
Dissolved oxygen (mg L^{-1})	9.97	0.63	4.23	5.00	19.16
NO_3^--N (µg L^{-1})	2707.00	484.00	328.00	200.00	16633.00
PO_4^{3-} (µg L^{-1})	289.00	212.00	144.00	80.00	847.00
pH	7.896	0.118	0.798	6.89	9.88
Turbidity (FAU)	208.00	116.00	763.00	3.00	4318.00
Chlorophyll-*a* (µg L^{-1})	37.11	4.41	29.900	0.00	131.11
Ammonia	49.00	7.00	24.00	10.00	90.00
Nitrite	5.00	1.00	3.00	0.000	10.00

ST3	Mean	SE Mean	StDev	Min	Max
Depth (cm)	18.07	1.77	13.510	0.000	37.150
Conductivity (µS)	589.60	21.60	148.100	159.600	922.300
Temperature (°C)	13.34	0.93	6.352	2.690	24.273
Dissolved oxygen (mg L^{-1})	9.16	0.51	3.479	5.000	19.404
NO_3^--N (µg L^{-1})	2969.00	606.00	4156.00	233.00	23867.00
PO_4^{3-} (µg L^{-1})	411.00	81.00	557.00	120.00	3870.00
pH	7.84	0.097	0.663	6.833	10.395
Turbidity (FAU)	65.30	13.40	88.60	1.300	300.000
Chlorophyll-*a* (µg L^{-1})	40.81	4.720	32.35	7.55	143.07
Ammonia	70.30	11.00	37.00	30.00	137.00
Nitrite	10.00	4.00	14.00	0.00	43.00

Table V.2: Basic descriptive physicochemical statistics for individual ponds across individual study year. Mean values are highlighted in bold.

13/14	**ST1**		**ST2**		**ST3**	
	Mean	StDev	Mean	StDev	Mean	StDev
Depth (cm)	**14.53**	7.32	**11.17**	9.71	**17.07**	14.55
Conductivity (µs)	**534.40**	147.30	**609.30**	176.80	**633.80**	127.10
Temperature (°C)	**12.49**	6.27	**11.45**	5.78	**11.83**	6.01
Dissolved Oxygen (mg L^{-1})	**8.88**	1.80	**6.42**	1.05	**6.45**	1.22
NO_3^--N (µg L^{-1})	**6000.00**	5790.00	**2951.00**	3121.00	**1995.00**	1852.00
PO_4^{3-} (µg L^{-1})	**947.00**	1134.00	**292.60**	128.90	**476.00**	812.00
pH	**7.31**	0.54	**7.46**	0.41	**7.55**	0.42
Turbidity (FAU)	**169.90**	200.10	**423.00**	1126.00	**74.60**	107.30
Chlorophyll-*a* (µg L^{-1})	**67.10**	59.20	**39.12**	27.81	**42.35**	34.06
14/15						
Depth (cm)	**15.35**	7.26	**12.68**	8.93	**20.06**	13.51
Conductivity (µs)	**453.50**	122.70	**496.50**	75.60	**493.60**	115.00
Temperature (°C)	**14.65**	6.56	**14.41**	7.74	**14.38**	7.55
Dissolved Oxygen (mg L^{-1})	**10.00**	2.56	**11.44**	4.37	**9.74**	2.80
NO_3^--N (µg L^{-1})	**6030.00**	6990.00	**3545.00**	4024.00	**5350.00**	5950.00
PO_4^{3-} (µg L^{-1})	**576.30**	417.80	**287.50**	180.00	**422.50**	290.90
pH	**7.78**	0.79	**8.26**	0.84	**7.95**	0.50
Turbidity (FAU)	**136.90**	204.70	**47.30**	73.10	**74.00**	78.40
Chlorophyll-*a* (µg L^{-1})	**46.45**	36.34	**44.41**	36.97	**46.13**	33.14
15/16						
Depth (cm)	**12.02**	7.50	**9.24**	7.33	**16.40**	11.88
Conductivity (µs)	**599.70**	181.50	**536.60**	117.64.96	**593.30**	178.20
Temperature (°C)	**12.11**	4.12	**12.80**	4.96	**12.52**	4.74
Dissolved Oxygen (mg L^{-1})	**11.48**	3.00	**12.14**	3.73	**11.84**	3.60
NO_3^--N (µg L^{-1})	**630.00**	322.70	**762.00**	409.00	**793.00**	516.00
PO_4^{3-} (µg L^{-1})	**804.00**	926.00	**752.00**	945.00	**739.00**	951.00
pH	**7.49**	0.44	**7.89**	0.83	**7.98**	0.91
Turbidity (FAU)	**26.08**	21.08	**17.46**	10.38	**19.50**	17.11
Chlorophyll-*a* (µg L^{-1})	**19.04**	18.59	**22.56**	17.95	**27.77**	26.96

Chlorophyll-*a* concentrations displayed distinct seasonal and temporal patterns throughout the study period, displaying an overall range of 0.00 to 276.66 µg L^{-1}. Winter months displayed relatively low concentrations, increasing slowly throughout the spring and fluctuating prior to substantial increase over the transition from spring to summer. Concentrations are high throughout summer months but are limited in ST2 and ST3 by summer dry-phases. Chlorophyll-*a* concentrations also displayed sharp increases upon pond rewetting, reaching relatively high concentrations throughout autumn months. Autumn periods are followed by a subsequent and often sharp decrease over the transitional period into winter as lower temperatures and sunlight restrict photosynthetic activity.

3.2.3 Nitrates (NO_3^--N)

Figure V.3 highlights NO_3^--N concentrations observed for individual ponds across the study. Variations were observed between ponds particularly in ST1, which displayed on average higher NO_3^--N concentrations (5014 ± 6093 µg L^{-1}) than ST2 (2707 ± 328 µg L^{-1}) and ST3 (2969 ± 4156 µg L^{-1}).

Seasonal and temporal patterns in NO_3^--N concentrations were also apparent, displaying higher concentrations in summer and autumn months, as well as sharp increases upon pond rewetting after dry phases. However, a substantial degree of variation existed between individual ponds and between study years.

3.2.4 Phosphate (PO_4^{3-})

PO_4^{3-} concentrations observed across individual ponds throughout the sampling period are displayed in figure V.3. PO_4^{3-} variations varied substantially across the 3 ponds. Particularly in ST1, which displayed distinctly elevated concentrations of PO_4^{3-}, on average 688 ± 799 µg L^{-1}in comparison to ponds ST2 (289 ± 144 µg L^{-1}) and ST3 (411 ± 557 µg L^{-1}). Concentrations in ST2 and ST3 were more comparable yet still exhibited a large degree of variation from one another.

Ponds displayed seasonal and temporal variations, apparent in the fluctuation of PO_4^{3-} concentrations throughout the study period, with an overall range of 80 – 4480 µg L^{-1}. However, seasonal fluctuations were not uniform across all 3 ponds, with elevated concentrations associated with pond rewetting after dry-phase and summer drawdown periods where ponds still held small quantities of water during warmer months.

Figure V.2: Depth and Chlorophyll-*a* results across the study period. Underlined points on the bottom axis represent points where one or more ponds were dry.

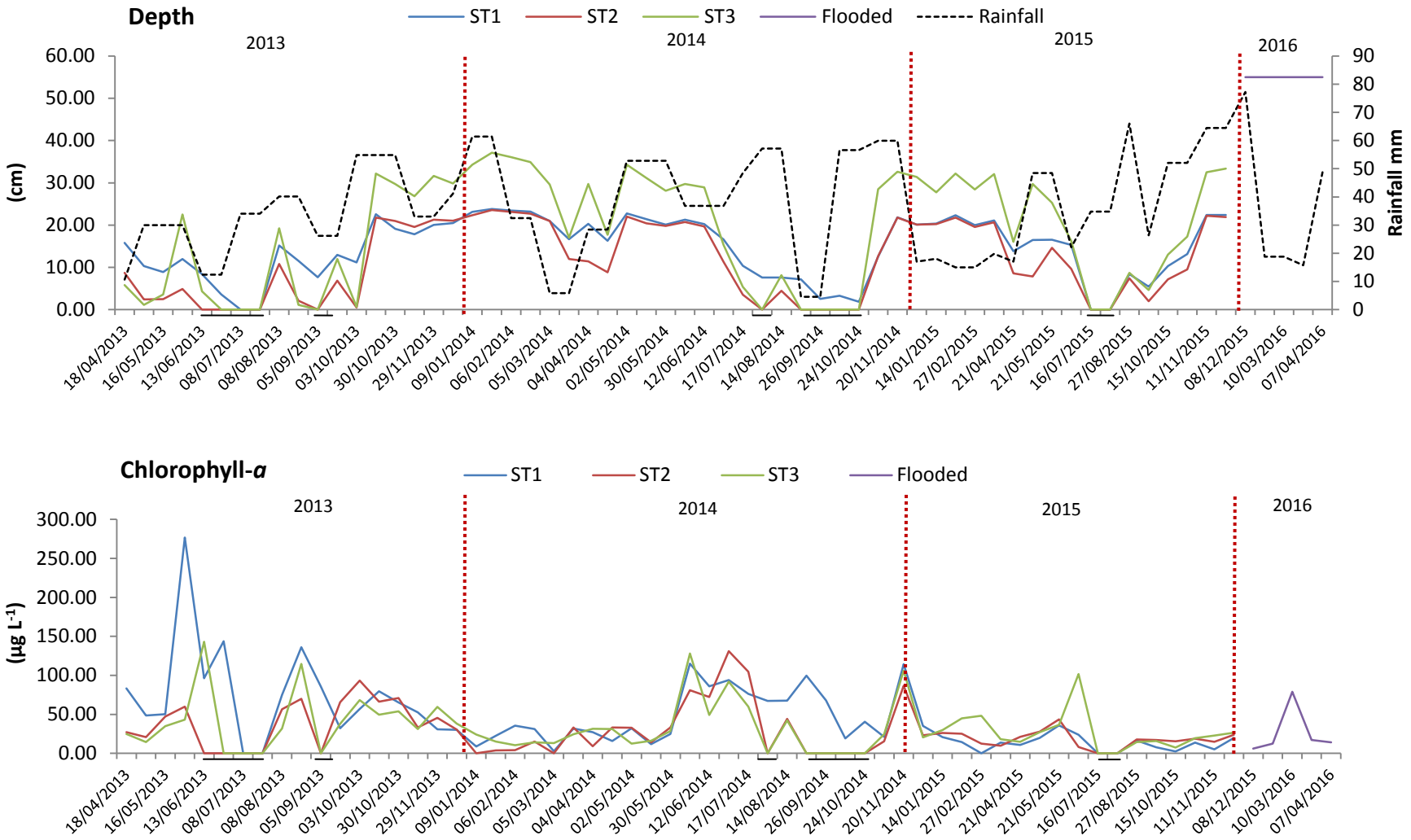
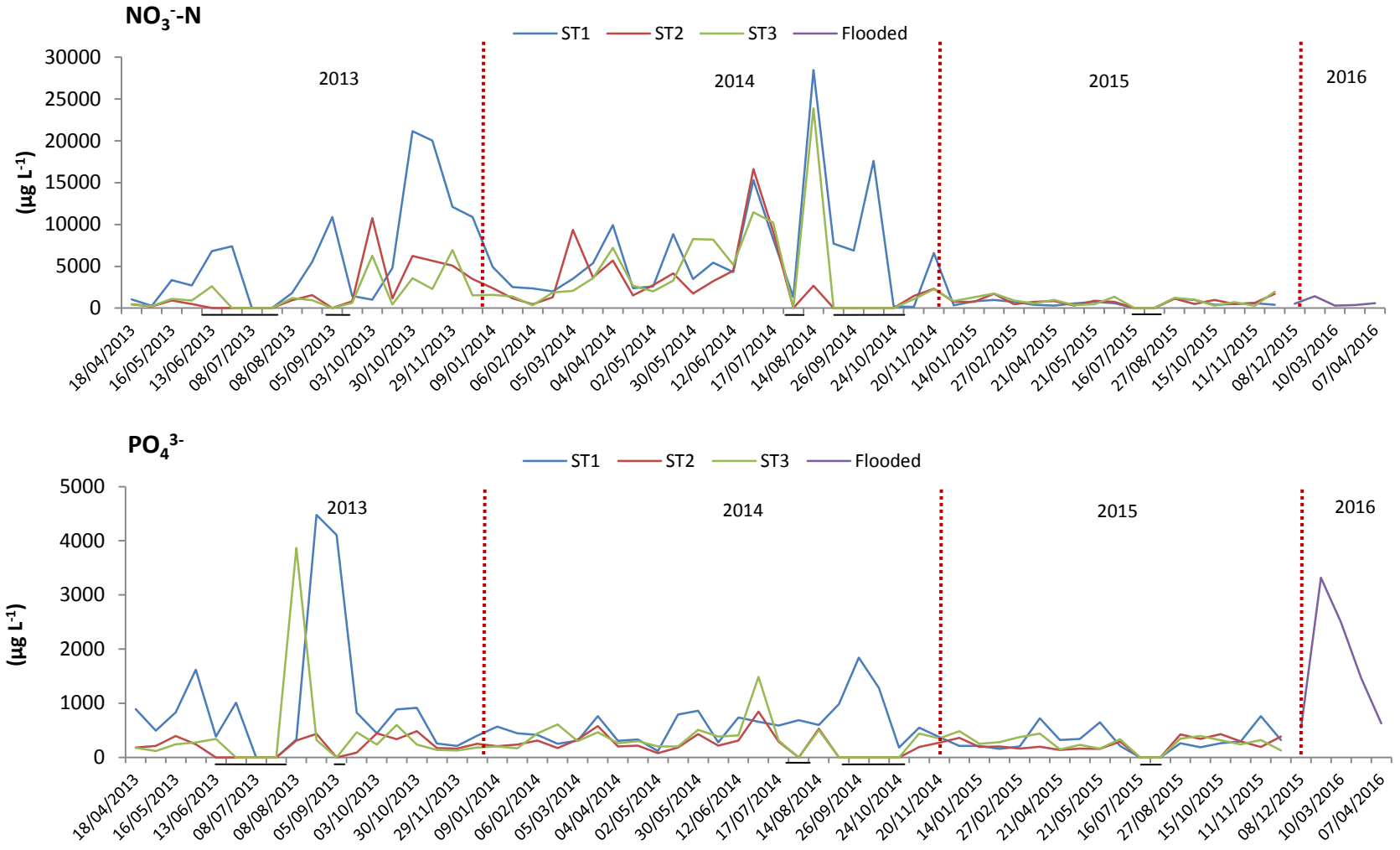


Figure V.3: Nitrate and Phosphate results across the study period. Underlined points on the bottom axis represent dry-phases for one or more ponds.



3.2.5 Basic physicochemical variables

Figures V.4 highlights concentrations for pH, Dissolved Oxygen, Conductivity and Temperature observed throughout the study period. Results demonstrate that ponds generally follow similar seasonal and annual physicochemical patterns, particularly across temperature. However, subtle variations exist between ponds suggesting a degree of physicochemical heterogeneity between ponds.

3.2.6 Ammonia (NH_3)

NH_3 concentrations observed across individual ponds over the sampling period are displayed in figure V.5. The overall range observed across the study period was 0.00 to 966.00 µg L^{-1}, although concentrations varied both seasonally and between ponds. Variations between ponds were considerable, ST1 displayed an overall mean of 72 µg L^{-1} whereas ST2 was 0.049 µg L^{-1}, ST3 was more comparable to ST1, with a mean of 70.0 µg L^{-1}.

3.2.7 Nitrite (NO_2^-)

Figure V.5 highlights NO_2^-concentrations observed over the sampling period. Overall range observed was 0.00 to 43.00 µg L^{-1}. Nitrite concentrations also displayed considerable seasonal variation and inter pond heterogeneity. Mean concentrations observed over the sampling period were 8.00, 5.00 and 10.00 µg L^{-1} for ST1,ST2 and ST3 respectively.

3.3 Depth, NO_3^--N, PO_4^{3-} and Chlorophyll-*a* Dynamics

Figure V.6 displays depth, NO_3^--N, PO_4^{3-} and chlorophyll-*a* variables for individual ponds. The ponds follow broadly similar patterns in response to seasonal changes, annual variation and particularly in response to dry phase and rewetting. As depth decreases in summer drawdown before drying, nutrient and chlorophyll-*a* concentrations become magnified due to decreased dilution. Elevated concentrations are also observed upon pond rewetting, potentially as nutrients are deposited or re-mobilised. Variation between the ponds were also observed with ST2 and ST3 behaving more similarly than ST1.

Figure V.4: Conductivity, Dissolved Oxygen, Temperature and pH results across the study period. Underlined points on the bottom axis represent points where one or more of the ponds were dry.

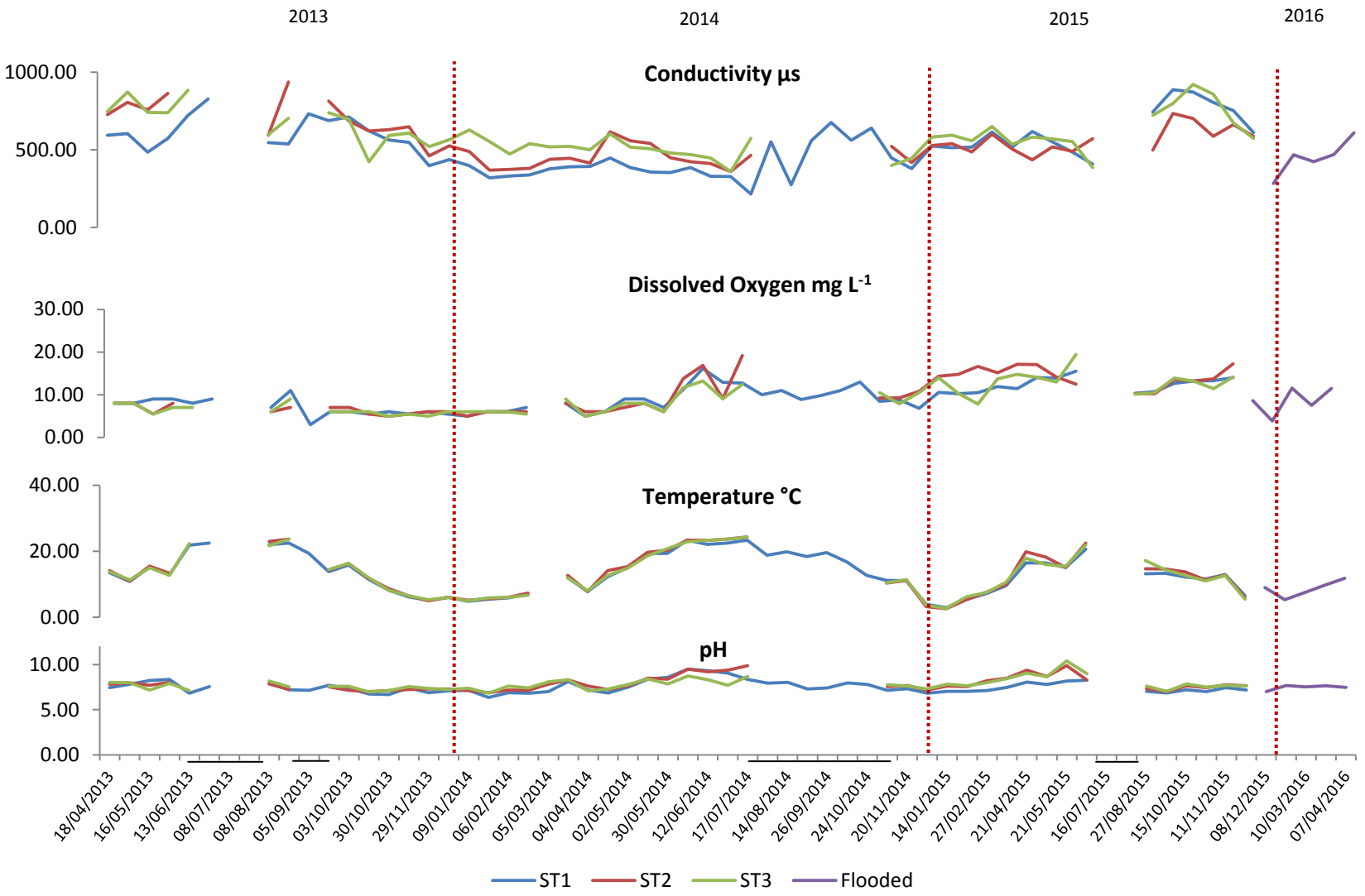


Figure V.5: Ammonia and Nitrite results across the study period. The purple line represents samples taken when the whole site was flooded.

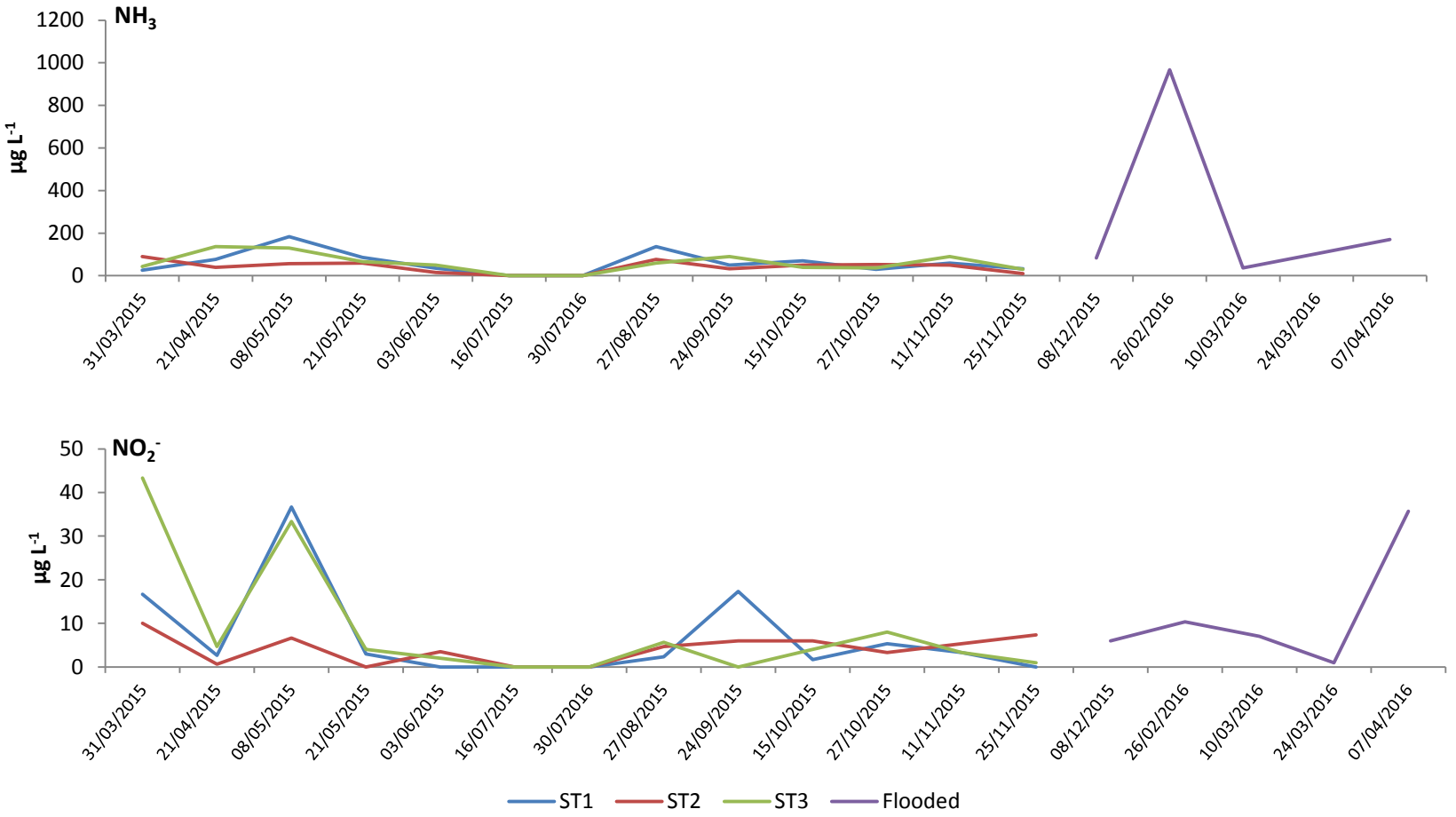
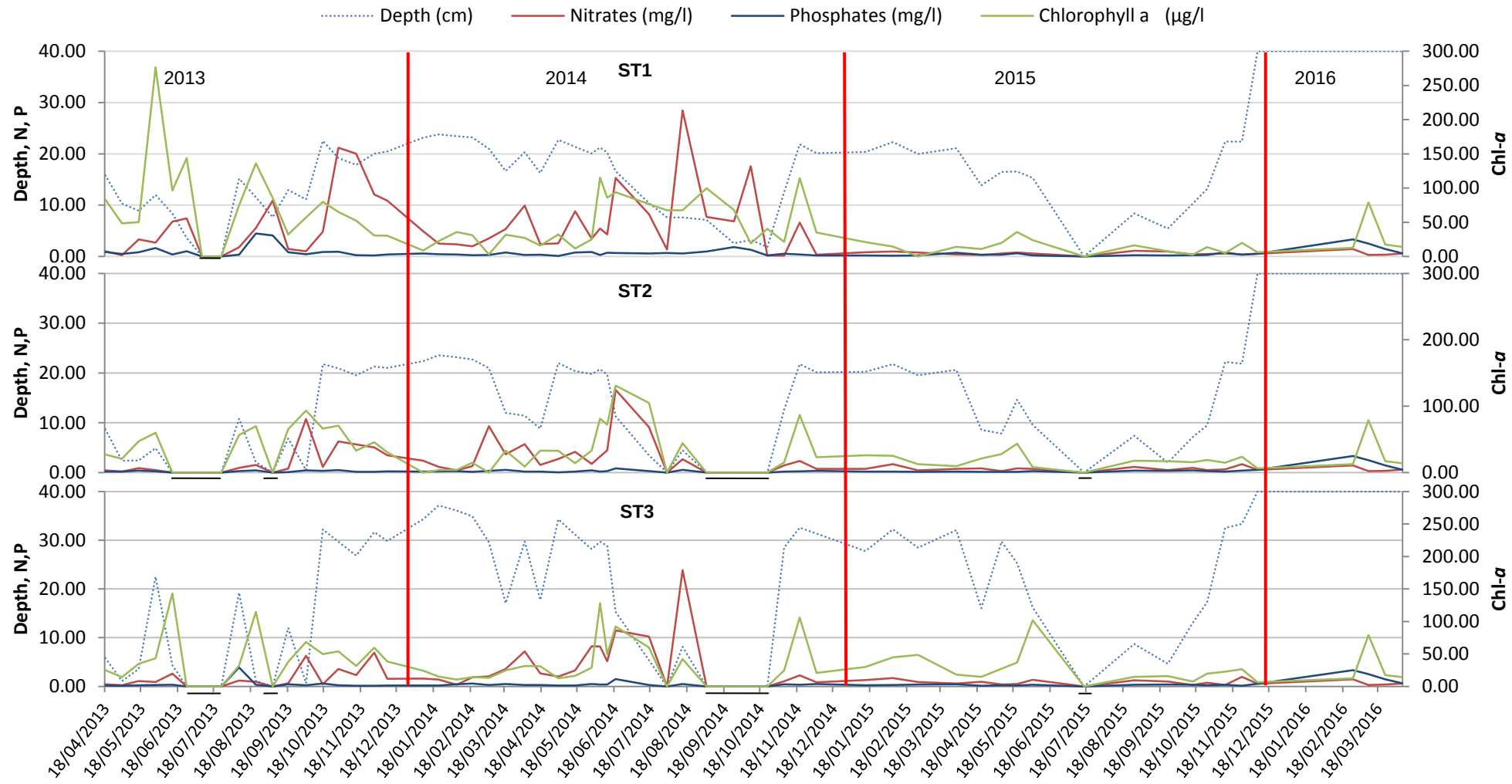


Figure V.6: Depth, Nitrate, Phosphate and Chlorophyll-*a* dynamics across the study period split by individual ponds. Underlined points on bottom axis represent dry-phases.



3.4 Statistical Analysis

3.4.1 PCA

PCA ordination plots for physicochemical variables across the full study period are displayed in figure V.7. Percentage variance explained by the first two axis was high (70.51%). The ordination shows distinct separation between physicochemical variables, individual ponds, hydro-period, years and season.

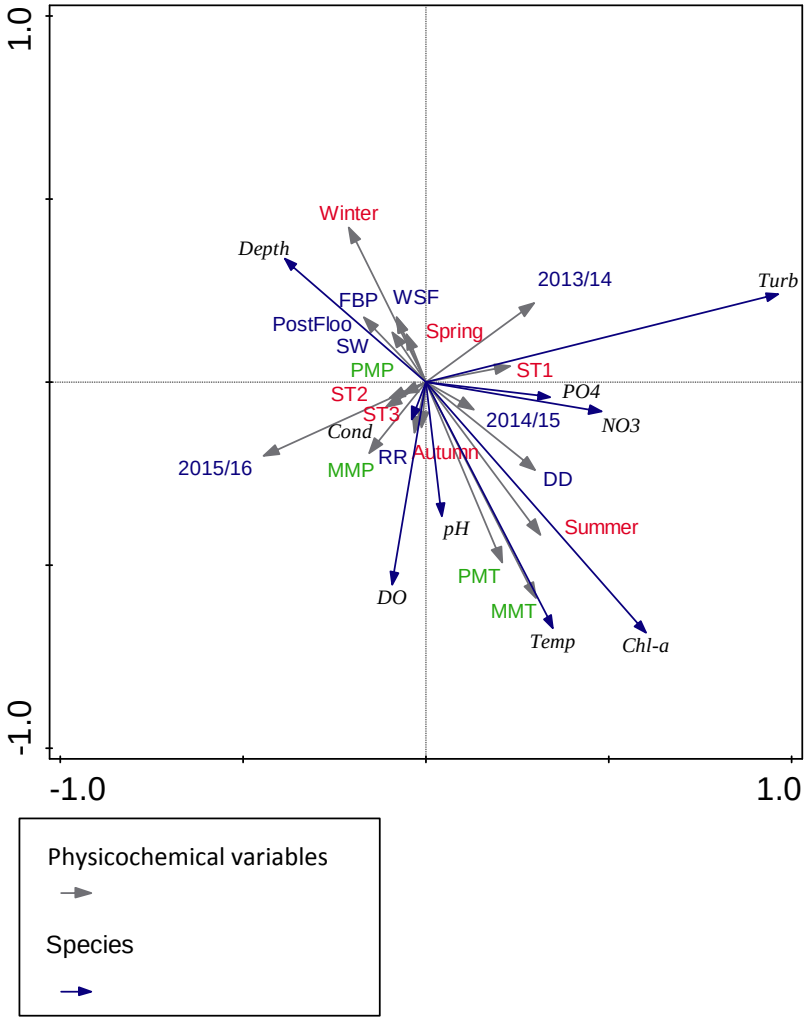
Figure V.8 displays species-sample ordinations and species-environment ordinations for individual sample years. Species relates to physicochemical variables. These have been included to highlight variations observed in the physicochemical dynamics across individual study years, exploring the separation between years observed in figure V.7.

3.4.2 Pearson's correlation

Correlation analysis was performed to assess relationships between physicochemical variables. Table V.3 highlights statistically significant correlation observed between physicochemical variables across all three ponds. Results confirm that both NO_3^--N and PO_4^{3-} are significantly correlated to chlorophyll-*a*. Other physicochemical variables were also significantly correlated to chlorophyll-*a*.

Tables V.4, V.5 and V.6 display Pearson's correlation results for individual ponds. The results highlight similar dynamics and idiosyncratic differences in the physicochemical functioning between ponds. Results also suggest that besides temperature, NO_3^--N and PO_4^{3-} were had the most significant impact on productivity, though this was less apparent in ST3. Variables displaying the most idiosyncratic behaviour in their relationships between the ponds were NO_3^--N, PO_4^{3-} and chlorophyll-*a.*

Figure V.7: PCA ordinations with environmental variables overlain for the whole dataset.



Environmental Variables		
Hydroperiod		
Standing Water	SW	Holding more than 10cm of water
Drawdown	DD	Less than 10cm depth
Recharge and refill	RR	Refill after drying
Flooded beyond pond	FBP	
Whole site flooded	WSF	
Climate		
Previous month Temperature	PMT	
Mean monthly Temperature	MMT	
Mean monthly precipitation	MMP	
Previous month precipitation	PMP	

Figure V.8: PCA ordinations with environmental variables overlain for data taken from individual years; a) 2013/14, b) 2014/15. c) 2015/16. WSF – (Whole site flooded). See previous figure V.7 for a tabular breakdown of variables used.

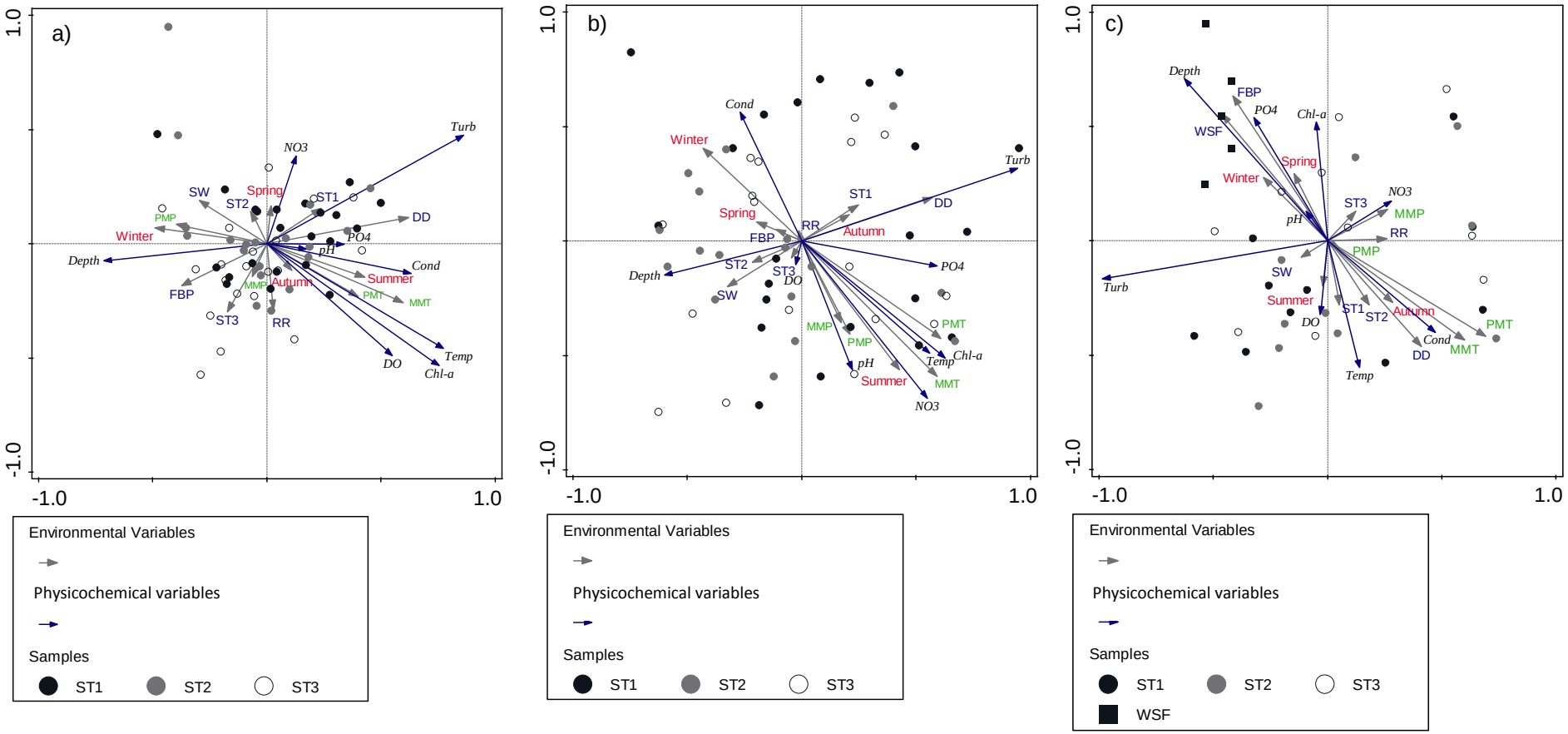


Table V.3: Pearsons Correlation Physicochemical Variables. All 3 ponds combined.

	Depth	Cond	Temp	DO	NO_3^--N	PO_4^{3-}	pH	Turb	Chl-*a*
Depth									r-value p-value
Cond	0.311 0.000								
Temp		0.54 0.000							
DO	0.372 0.000	0.576 0.000	0.615 0.000						
NO_3^--N			0.359 0.000						
PO_4^{3-}		0.245 0.001	0.403 0.000		0.289 0.000				
pH	0.546 0.000	0.771 0.000	0.734 0.000	0.796 0.000	0.297 0.000	0.281 0.000			
Turb	-0.191 0.014	0.322 0.000	0.407 0.000		0.296 0.000	0.488 0.000	0.226 0.003		
Chl-*a*		0.321 0.000	0.576 0.000	0.285 0.000	0.384 0.000	0.424 0.000	0.437 0.000	0.461 0.000	

Table V.4: ST1 Pearsons Correlation Physicochemical Variables.

ST1	Depth	Cond	Temp	DO	NO_3^--N	PO_4^{3-}	pH	Turb	Chl-*a*
Depth									r-value p-value
Cond									
Temp		0.359 0.006							
DO		0.455 0.000	0.565 0.000						
NO_3^--N									
PO_4^{3-}			0.421 0.001						
pH	0.476 0.000	0.548 0.000	0.669 0.000	0.715 0.000					
Turb	-0.415 0.002	0.383 0.004	0.46 0.000			0.573 0.000			
Chl-a			0.484 0.000			0.482 0.000	0.347 0.008	0.432 0.001	

Table V.5: ST2 Pearson's Correlation Physicochemical Variables.

ST2	Depth	Cond	Temp	DO	NO_3^--N	PO_4^{3-}	pH	Turb	Chl-*a*
Depth									r-value
									p-value
Cond	0.374								
	0.004								
Temp		0.648							
		0.000							
DO	0.455	0.599	0.616						
	0.000	0.000	0.000						
NO_3^--N			0.417						
			0.001						
PO_4^{3-}	0.302	0.585	0.636	0.449	0.593				
	0.021	0.000	0.000	0.000	0.000				
pH	0.615	0.832	0.775	0.81	0.395	0.675			
	0.000	0.000	0.000	0.000	0.000	0.000			
Turb		0.438	0.432		0.481	0.395	0.284		
		0.001	0.001		0.000	0.003	0.036		
Chl-*a*		0.471	0.67	0.372	0.64	0.615	0.55	0.554	
		0.000	0.000	0.004	0.000	0.000	0.000	0.000	

Table V.6: ST3 Pearson's Correlation Physicochemical Variables.

ST3	Depth	Cond	Temp	DO	NO_3^--N	PO_4^{3-}	pH	Turb	Chl-*a*
Depth									r-value
									p-value
Cond	0.393								
	0.002								
Temp		0.55							
		0.000							
DO	0.456	0.632	0.645						
	0.000	0.000	0.000						
NO_3^--N			0.497	0.497					
			0.000	0.000					
PO_4^{3-}			0.419						
			0.001						
pH	0.632	0.828	0.732	0.822	0.323	0.311			
	0.000	0.000	0.000	0.000	0.014	0.018			
Turb		0.274	0.377		0.33		0.302		
		0.043	0.005		0.014		0.025		
Chl-*a*		0.371	0.641	0.394	0.371		0.483	0.362	
		0.004	0.000	0.002	0.004		0.000	0.007	

3.4.3 Climate and hydrological dynamics

Stepwise regression was performed with climate variables and depth to determine climatic variables, which create the best model of predicting pond depth and essentially hydro-period. Table V.7 highlights results for all three ponds across the full study period, to assess broad climatic effects on pond depth. Percentage explanation was poor across both models with model 1 and 2 displaying respective R^2 values of 0.410 and 0.552, based on mean 1 week temperature and 3 weeks cumulative rainfall.

Table V.7: Stepwise regression analysis for all ponds across all years.

	All 3 Ponds across all years		
Model	Variables	R^2	sig
1	TEMP1WEEK	0.410	0.000
2	RAIN3WEEK	0.552	0.000

Table V.8 displays stepwise regression results for individual ponds to assess the degree to which depth of individual ponds was could be explained by climate. Results show a greater degree of explanation within the models with maximum R^2 values for ponds ST1, ST2 and ST3 ranging from 0.620 to 0.648. The model for ST2 and ST3 gave the same set of predictor variables (mean weekly temperature and 3 weeks cumulative rainfall), albeit slightly different degrees of explanation in R^2 values. ST1 depth was better explained by weekly temperature and 2 weeks cumulative rainfall.

Table V.8: Stepwise regression analysis for individual ponds across all years

	Individual Ponds								
	ST1			ST2			ST3		
Model	Variables	R^2	sig	Variables	R^2	sig	Variables	R^2	sig
1	TEMP1WEEK	0.494	0.000	TEMP1WEEK	0.445	0.000	TEMP1WEEK	0.477	0.000
2	RAIN2WEEK	0.620	0.000	RAIN3WEEK	0.648	0.000	RAIN3WEEK	0.636	0.000

Table V.9 highlights stepwise regression results for individual ponds across individual study years. Depth in individual ponds was better explained across this analysis with R^2 values ranging from 0.748 to a maximum of 0.905. This table highlights variations between ponds, with ST2 and ST3 showing more similarity than ST1 and also differences in the impact of certain climate variables on depth between years.

Table V.9: Stepwise regression analysis for individual ponds across individual study years. Variables highlighted in red have been omitted from the model.

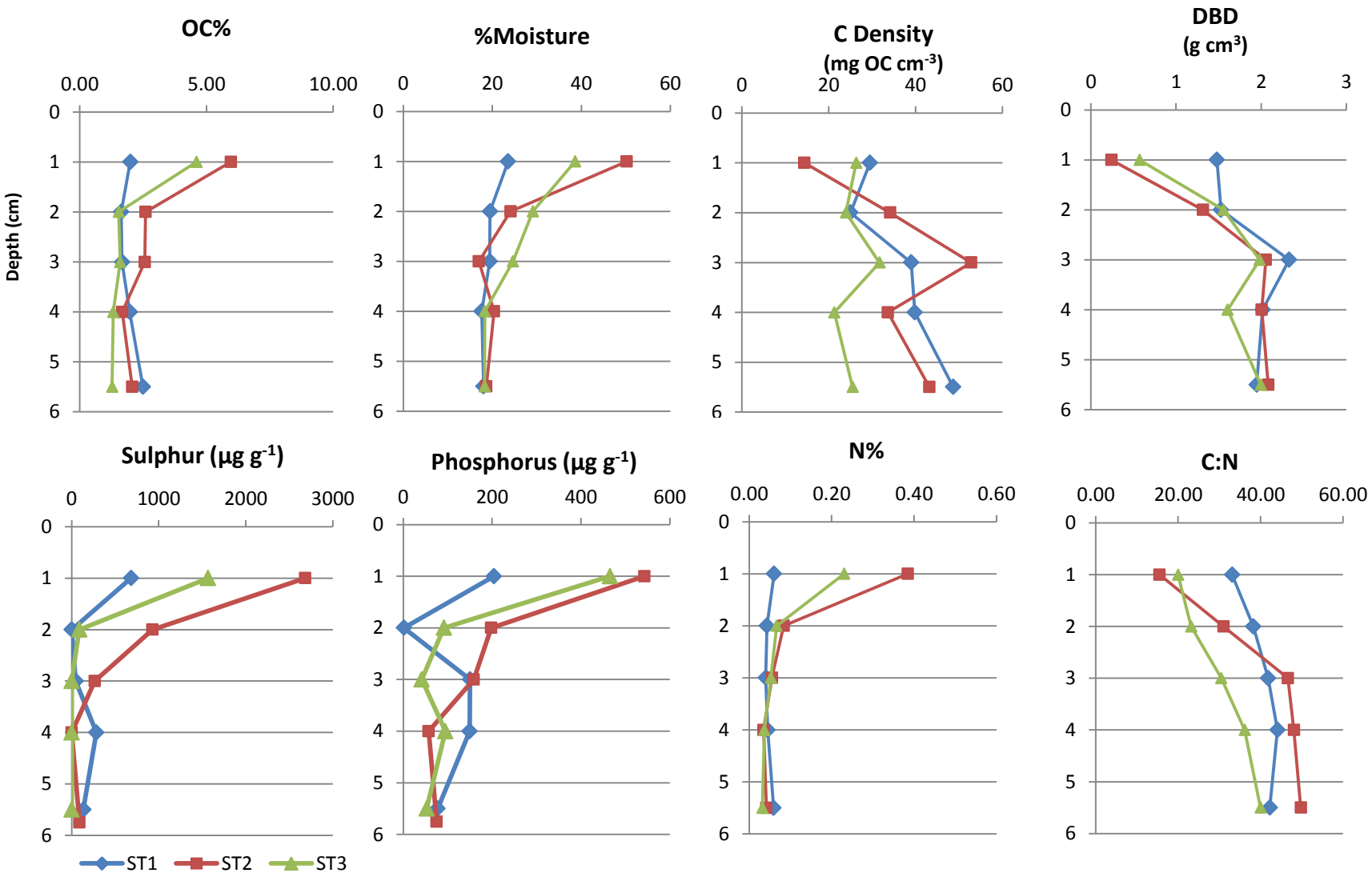
Individual Ponds and Years									
2013/14									
	ST1			ST2			ST3		
Model	Variables	R^2	sig	Variables	R^2	sig	Variables	R^2	sig
1	RAIN3MONTH	0.740	0.000	RAIN3MONTH	0.724	0.000	RAIN3MONTH	0.583	0.000
2	TEMP1WEEK	0.828	0.000	TEMP2MONTH	0.790	0.000	TEMP1WEEK	0.644	0.000
3	RAIN2WEEK	0.862	0.000	TEMP6MONTH	0.846	0.000	RAIN3WEEK	0.726	0.000
4				(- RAIN3MONTH)	0.832	0.000	(-RAIN3MONTH)	0.719	0.000
5				RAIN2WEEK	0.889	0.000	RAIN4WEEK	0.762	0.000
6							TEMP6MONTH	0.827	0.000
2014/15									
1	TEMP3MONTH	0.665	0.000	TEMP2MONTH	0.550	0.000	TEMP2MONTH	0.580	0.000
2	RAIN5WEEK	0.831	0.000	RAIN5WEEK	0.817	0.000	RAIN5WEEK	0.757	0.000
2015/16									
1	TEMP1WEEK	0.659	0.001	TEMP1WEEK	0.430	0.021	TEMP1WEEK	0.712	0.001
2	RAIN2WEEK	0.789	0.001	RAIN2WEEK	0.748	0.002	RAIN2WEEK	0.841	0.000
3	TEMP2WEEK	0.880	0.000				RAIN4WEEK	0.905	0.000
4	(-TEMP1WEEK)	0.874	0.000						

3.5 Sediment Core Analysis

3.5.1 Physical and chemical variables

Results from the sediment core analysis show similar down core patterns to those extracted from the mature experimental ponds. However, it appears that the amount of sediment accumulated is limited to the top 1 cm. Figure V.9 displays physical and chemical variables observed across all three ponds. ST2 and ST3 displayed a profile more characteristic of sediment, with decreasing OC% and increasing DBD with depth, similar to the mature ponds in chapter IV. This was less apparent in ST1, which displayed fairly consistent levels, suggesting that the developing sediment layer in ST1 was not as substantial as the other two ponds.

Figure V.9: Sediment core results for the experimental ponds.



3.5.2 Carbon Storage

Table V.10 displays estimated OC storage values based on the top 1 cm and 2 cm of the core calculated using the formula outlined in chapter III. Respective burial rates are also displayed to allow for comparison to mature ponds. In order to be conservative with estimates, it was accepted that the top 1 cm of ponds ST2 and ST3 were likely to be early stage sediment, with respective OC storage values of 24.21 and 57.24 g OC m^{-2}. OC Storage in the top cm of ST1 was estimated to be 147.92 g OC m^{-2} markedly elevated beyond the other two ponds. Results from the sediment physicochemical analysis (figure V.9), suggest this layer is uncharacteristic of sediment and similar to the underlying clay soil, indicating a limited development of an initial sediment layers in this pond. Results for ST1 have therefore been omitted from the study.

Table V.10: Carbon Storage and burial results from sediment cores taken from the newly constructed ponds.

Pond	Sediment Depth (cm)	Carbon Storage (g OC m^{-2})	Burial Rate (g OC m^{-2} yr^{-1})
ST2	1	24.21	8.07
	2	155.78	51.93
ST3	1	57.24	19.08
	2	213.04	71.01

4.0 Discussion

4.1 Pond Hydrology

The ponds are situated on a deep layer of clay and are filled during rainfall events, surface run-off and horizontal through-flow, between top soil and impermeable clay layers. Depth appeared strongly affected by rainfall (Figure V.2). The ponds were deepest in winter, often flooded beyond boundaries under higher rainfall. Sustained dry periods resulted in summer and autumn dry phases, only refilling after subsequent rainfall events. However, the impact of rainfall events on an individual ponds depth and hydro-period displayed clear variation between ponds.

Results from the stepwise regression analysis (Tables V.7) shows that depth for all 3 ponds across the study period was best described by mean weekly temperature and cumulative 3 week rainfall ($R^2 = 0.552$). Accounting for individual ponds (Table V.8) and individual study years (Table V.9), results in a considerably larger degree of explanation for pond depth. Table V.8 highlights subtle variations between ponds. For instance, depth in ST2 and ST3 was best described by mean weekly temperature and cumulative 3 week rainfall, whilst ST1 was best described by mean weekly rainfall and cumulative 2 week rainfall. These results likely reflect the individual ponds location in the field and its relationship to the local water table.

Considering individual ponds across separate study years (Table V.9), produced the best degree of explanation for pond depth. This analysis further highlighted differences in climate-hydrological interactions between ponds. This was most striking across the 2013/14 study year, where climate variables best describing pond depth showed the greatest degree of dissimilarity between ponds, besides cumulative 3 month rainfall, which was the most significant variable observed across all ponds. Variation was also apparent across 2014/15 with depth in ST2 and ST3 best described by mean 2 month temperature and cumulative 5 week rainfall, whilst depth in ST1 was best described by mean 3 month temperature and cumulative 5 week rainfall.

Variations were less apparent across 2015/16, with depth in ponds best described by mean weekly temperature and cumulative 2 week rainfall. The only differences observed being the inclusion of mean 2 weekly temperature in ST1 and cumulative 4 week rainfall in ST3. This may be a result of limited dry-phases or the extreme weather event (Storm Desmond) observed across winter in this study year, which resulted in the whole site flooding, reducing the resolution of observed depth changes between individual ponds across sample weeks. The site manager (Alex Lister, NWT) has speculated that the drainage of the site was altered during this event resulting in

sustained flooding of the field to the present day, an interesting occurrence, highlighting the impact that extreme weather events can have on ponds and small water bodies.

Variations between ponds across the same year, likely reflect the location of individual ponds. For example across the year 2013 all ponds were subject to summer dry-phases. ST2 and ST3 were also subject to subsequent dry-phases in the autumn. However, hydro-period was not uniform across all ponds (see figure V.10). ST2 was the first to dry on 14-06-13, followed by ST3 on 25-06-13 and finally ST1 on the 08-07-13. All ponds remained dry prior to refill on 08-08-13. Variations existed across later hydro-periods. ST2 and ST3 were subject to substantial drawdown, barely holding water, on the 22-08-13. By early autumn 05-09-13, they were completely dry, refilling after rainfall on 19-09-13 and nearly drying out completely again on 03-10-13. Throughout this period ST1 still held water, although depth did decrease to fairly low levels, prior to complete refilling of all the ponds on 17-10-13. ST1 displayed similar behaviour in later years, holding water whilst the other ponds dried completely.

Figure V.10: The three ponds across the same sampling date 14-06-13 displaying variations in summer hydrology.

Annual variations in pond drying were also observed. For instance, ST2 and ST3 were subject to a much later summer dry-phase in 2014, drying out briefly on 01-08-14 until

refill on 14-08-14. These ponds had dried again completely by 02-09-14 and were subject to a sustained dry-phase throughout the autumn, prior to refill on 07-11-14. In 2015 the ponds did not dry completely, holding low levels of water from 16-07-15 up until around 27-10-15.

Hydrological variability between ponds followed similar patterns to previous studies (Jeffries, 2008). It was suggested to be the result of the individual ponds situation within the field in relation to surface run-off and local water table. Hydrology also played a key deterministic role in the establishment, succession and heterogeneity of vegetation communities across ponds. It was speculated that hydrological driven physicochemical gradients across the site, potentially resulted in faster successional progression and ultimately terrestrialisation of the ponds, evident in the earlier establishment of *L.riparium*, *E.palustris* and *G.fluitans*.

Results suggest that climate plays an important role in determining pond depth and ultimately hydro-period. Variations in climate between years drive different patterns of seasonal dry-phases and recharge events. Results also confirm the importance of an individual ponds precise location in governing its hydrological behaviour and susceptibility to local climate. Micro-scale variations are a factor that must be considered when attempting to assess the impacts of climate change upon the functioning of small ponds and similar ecosystems across regional scales.

4.2 Nutrients

NO_3-N concentrations were high in comparison to other aquatic ecosystems (see Table V.11), with levels of up to 28470 µg L^{-1} observed over the study period (Figure V.3). Concentrations also varied considerably between ponds (mean 2707 – 5014 µg L^{-1}) and were generally highest in ST1, suggesting a spatial element in the delivery and proportion of NO_3-N entering the pond. Intra-annual variability in NO_3-N concentrations is clearly evident in figure V.3 and high standard deviation in table V.2. In ST2 and ST3, NO_3-N were positively correlated to temperature, suggesting seasonal influences or reflecting climate driven hydro-periods in these ponds, opposed to the markedly different hydro-period behaviour of ST1. Figure V.3 demonstrates that increasing NO_3-N concentrations closely follow rainfall events, particularly when rewetting after dry-phases. This suggests that either surface run-off drives NO_3-N delivery into the system or rewetting drives remobilisation of NO_3-N from sediment back into the water column (Reverey et al., 2016). It is also possible that these processes occur simultaneously.

Table V.11. Nitrate, Phosphate and Chlorophyll-*a* concentrations across a variety of waterbodies. * indicates values reported are maximum or snapshot summer values.

Water Body Type	Chl-*a* (µg L^{-1})	NO_3^--N (µg L^{-1})	PO_4^{3-} (µg L^{-1})	N:P:Chl-*a*	Reference
ST1	49.97	5014.00	688.00	**100:14:1**	
ST2	37.11	2707.00	212.00	**73:6:1**	
ST3	40.81	2969.00	411.00	**73:10:1**	
Mean this study	***43.24***	***3563.00***	***437.00***	***82:10:1***	**This Study**
Mean Summer Values					
ST1	138.62	5583.60	794.17	40:6:1	
ST2	72.96	3058.67	376.10	42:5:1	
ST3	124.30	4886.23	821.90	39:7:1	
Mean this study	***111.96***	***4509.5***	***664.06***	***40:6:1***	This Study
Hypertrophic					
Urban landscape ponds, Poland	162.00	1288.00	87.00	8:0.5:1	Joniak, 2007*
Barton Broad Eutrophic shallow lake, England	130.38		15.17	x:0.1:1	Lau, 2002
Shallow Ponds, SE England	91.19	1845.16	91.16	20:1:1	Bennion & Smith 2000*
Peri-urban Ponds, Belgium	72.24		132.38	x:2:1	Teissier 2012
Ponds/Pools Brown Moss , NW England	49.38	449.00	389.09	9:8:1	Chaichana 2011
Experimental shallow lake mesocosms, NW England	43.59	265.71	125.00	6:3:1	Feuchtmayr, 2009
Eutrophic					
Pond vegetated zones, Poland	32.39		65.00	x:2:1	Joniak, 2007*
Agricultural ponds, Poland	11.00	1284.00	88.00	117:8:1	Joniak, 2007*
Seasonal Turloughs Ireland	7.22		8.05	x:1:1	Pereira et al., 2010
Mesotrophic					
Clay Pits, Poland	4.00	775.00	51.00	193:13:1	Joniak, 2007*
Oligotrophic					
Mid-forest ponds, Poland	3.00	859.00	10.00	286:3:1	Joniak, 2007*

PO_4^{3-} concentrations were also high in all ponds in comparison to other aquatic systems (see table V.11). Similar to NO_3-N, variations were also observed between ponds. ST1 displayed distinctly elevated concentrations of PO_4^{3-}, on average 688 ± 799 µg/l, with maximum concentrations reaching 44800 µg L^{-1}, in comparison to ponds ST2 (289 ± 212 µg L^{-1}) and ST3 (411 ± 557 µg L^{-1}). Intra-annual variability in phosphate concentrations were evident in figure V.3 and high standard deviation table V.2. PO_4^{3-} was positively correlated with temperature (<0.001) in all ponds suggesting seasonal and climatic influences on concentrations. Similar to NO_3-N, PO_4^{3-} concentrations

closely follow rainfall events, during both periods of standing water and upon rewetting after dry phase, confirming the importance of rainfall in nutrient delivery and remobilisation.

Increasing PO_4^{3-} concentrations also occur during periods of drawdown, where pond depth decreases. This is likely the result of decreasing dilution and resulting magnification of PO_4^{3-} loadings during these periods. Similar patterns of increase and decrease were also observed across spring 2014, potentially indicating utilisation between input events. Overall fluctuations of PO_4^{3-} display markedly more variability than NO_3-N, potentially indicating differing dynamics and processes of utilisation across individual ponds.

The ponds are located within a nature reserve and do not receive any agricultural run-off; the clay backfill in which the ponds were constructed also eliminates the possibility of groundwater upwelling to the ponds. Nutrient supply to the ponds is largely from direct deposition from animals or indirectly from the surrounding catchment via surface run-off and through -flow, following rainfall. Remobilisation of nutrients from the sediment upon rewetting will also be a considerable factor driving nutrient concentrations in these temporary features (Reverey et al., 2016).

Excrement and defecation from both avian and mammalian sources is often apparent (see figure V.11), with ponds and the surrounding field utilised across the year for habitat, feeding or bathing purposes by a number of species. Of particular significance is a heronry located in woodland adjacent to the field, and groups of 2-6 Grey Herons (*Ardea cinerea*) are regularly observed at the study site and their excrement is often evident both in the ponds themselves and throughout the surrounding catchment.

Figure V.11: Signs of avian defecation in the experimental ponds.

Avian faecal matter particularly that from water birds, is rich in nitrogen and phosphorus. Excrement from psicivorous species, such as Grey Herons, is substantially high in phosphorus (Table V.12). It is possible that faecal matter from this species contribute significantly to nutrient loadings within the ponds, through either direct deposition or run-off from the surrounding catchment. Rabbit (*Oryctolagus cuniculus*) droppings are also abundant throughout the catchment and are deposited directly in dry-phases, so likely contribute to nutrient loadings within the ponds.

Table V.12: Nutrient concentrations from faecal matter of animals observed at the Hauxley site and projected impact of 1g of faecal matter on pond nutrient concentrations based on mean water volume.

Species	N (mg g^{-1})	P (mg g^{-1})	Potential impact on N concentration (µg L^{-1})			Potential impact on P concentration (µg L^{-1})		
			ST1	ST2	ST3	ST1	ST2	ST3
Mallard	26.20	13.20	74.43	77.98	51.17	37.50	39.29	25.78
Starling	46.20	7.90	131.25	137.50	90.23	22.44	23.51	15.43
Gull	29.60	16.20	84.09	88.10	57.81	46.02	48.21	31.64
Heron	42.10	114.70	119.60	125.30	82.23	325.85	341.37	224.02
Rabbit	24.00	14.00	68.18	71.43	46.88	39.77	41.67	27.34

Data on birds taken from Marion et al., 1994. Rabbit data from Tabaro et al.,2012

The impact of birds on water quality in lakes has highlighted the potential for these inputs to contribute substantially to nutrient loading in these systems, often resulting in eutrophication of receiving waters (Chaichana et al., 2011; Boros et al., 2008). In comparison to larger water bodies, source inputs in ponds are magnified given lower water volume. Inputs of nutrient rich faecal matter will therefore have a considerable effect. The guano-trophication of small pools has been recently documented (Chaichana et al., 2011; 2010), demonstrating the potential for avian activity to become primary source of nutrients, impacting the trophic state of small water bodies.

NO_3-N concentrations were most comparable to agricultural and urban landscape ponds in Poland, (Joniak et al., 2007) and Brown Moss Pools UK (Chaichana et al., 2011; 2010), which are subject to inputs from agricultural and avian sources. Concentrations observed in this study were considerably higher than these values, likely arising from comparably lower water volume and magnification of nutrient inputs, such as heron faeces, as demonstrated in table V.12. Values represent mean pond volume and so do not reflect the increased magnification of NO_3-N concentrations as depth and dilution decrease. PO_4^{3-} concentrations were also most comparable to Brown Moss pools (Chaichana et al., 2011; 2010), elevated above published results for hyper-eutrophic urban, agricultural and shallow ponds (see table V.12). Considering the potential effect of Grey Heron faeces on PO_4^{3-} concentrations (table V.11), it

becomes clear that these birds have the potential to contribute significantly to observed PO_4^{3-} concentrations, with just one gram of faecal matter resulting in potential concentrations of between 224.02 – 341.37 µg L^{-1}.

Given such high NO_3-N and PO_4^{3-} concentrations, it is likely that nutrient loadings come from a combination of sources, including; animal faeces, degradation of OM within the system and remobilisation from sediment layers upon rewetting. Variations observed between ponds likely reflect these different source inputs, with discrepancies in the proportion of nutrients delivered to the ponds, driven by the individual ponds location and its relation to surface run off and these stochastic ecological inputs.

4.3 Primary Productivity

Chlorophyll-*a* concentrations varied widely across the dataset (0.00 – 276.66 µg L^{-1}) with an overall mean of 42.63 µg L^{-1}. All values were well within the range of the probes ability to produce accurate results (see figure III.2). Ponds displayed substantial spatial variation in mean chlorophyll-*a* concentrations (tables V.4, V.5 and V.6), with values of 49.97, 37.11 and 40.81 µg L^{-1}observed for ST1, ST2 and ST3 respectively. The range of chlorophyll-*a* concentrations observed for individual ponds was more variable with levels of 0.00 - 276.66, 0.00 - 131.11 and 7.55 - 143.08 µg L^{-1}observed throughout the study period for ST1, ST2 and ST3 respectively (figure V.2). This suggests substantial inter annual variability or seasonal fluctuation. Productivity also showed the capacity to vary substantially over the course of a single day. Figure V.12 highlights intense photosynthetic activity of an algal bloom as it floats to the surface from sampling at 11:30am to afternoon observations at roughly 1:30pm.

Figure V.12: Photosynthetic activity of an algal bloom across the course of a single sampling session. Left: Photo taken ~11:30am. Right: Photo taken at ~1:30pm.

Ponds exhibited nutrient concentrations, most comparable to those recorded in other shallow ponds and hyper-eutrophic pools, in lowland agricultural landscapes (Chaichana et al., 2011b; Bennion & Smith, 2000), and phytoplankton turbid lakes (Allende et al., 2009). According to the trophic classification of freshwater systems (Forsberg & Ryding 1980), all ponds would be classified as hyper-eutrophic, exhibiting chlorophyll-*a* concentrations >40 µg L^{-1}. Results confirm the disproportionate productivity associated with small ponds in comparison to larger aquatic systems, displaying comparable levels to shallow lowland ponds and pools (See table V.13 for comparative data from a range of aquatic habitats).

Contrary to this chlorophyll-*a* concentrations reported for phytoplankton turbid lakes, urban landscape and shallow ponds were above levels observed in the experimental ponds. However, values provided were snap shot values in late spring or summer, unrepresentative of mean annual concentrations. Summer values observed in this study were more comparable to those reported in other studies. Although, when comparing the range of these contrasting systems, we can see that shallow and urban landscape ponds have the capacity to be substantially more productive (table V.13), despite considerably lower nutrient concentrations (table V.11). This would suggest a rate limiting factor on productivity within the ponds other than nutrient concentrations.

Table V.13: Chlorophyll-*a* concentrations observed in this study compared to a variety of other waterbodies.

Water Body Description/Classification	Mean Chl-*a* (µg L^{-1})	Range	Reference
Urban Landscape Ponds	162.00		Joniak, 2007*
Mean this study (Summer)	**111.96**	**72.96 – 138.62**	**This Study**
Shallow Ponds	91.20	7.00 – 501.00	Bennion & Smith, 2000*
Phytoplankton-Turbid Lakes	68.75	14.20 – 125.70	Allende et al., 2009*
Hypereutrophic Pools	49.38	14.40 – 123.10	Chaichana., 2011
Mean this study	**43.24**	**2.80 – 276.66**	**This Study**
Mesotrophic lakes	34.10	0.80 – 135.00	Binhe et al., 2010
Mesotrophic Lakes	30.00		Tilahun & Ahlgren,2010
Eutrophic Pond	13.80	1.30 – 57.00	Nozaki et al., 2009
Inorganic Turbid Lakes		14.60 – 18.30	Allende et al., 2009*
Mesotrophic Lakes	8.50±0.90	0.40 – 46.60	Lee et al., 2010
	6.30±0.70	0.10 – 46.60	Lee et al., 2010
Clear Vegetated Lakes	2.15	1.60 – 2.70	Allende et al., 2009*

Chlorophyll-*a* was more consistent in its relationship to other variables across the ponds (Table V.4, V.5 and V.6). Across the combined data set chlorophyll-*a* displayed positive correlation to temperature, NO_3^--N, PO_4^{3-}, pH and turbidity. However, differences were observed between individual ponds, indicating a degree of idiosyncrasy in the relationships of chlorophyll-*a* to other physicochemical variables. Temperature showed strong positive correlation in all ponds, reflecting seasonal changes and increased concentrations observed throughout warmer months. However, other relationships were more idiosyncratic for instance positive correlation with PO_4^{3-}, pH and turbidity observed only in ST1 and ST2, whilst correlation to NO_3^--N existed only in ST2 and ST3.

Similar relationships to pH and turbidity were also observed across all three ponds, likely a result of photosynthetic processes. pH increases as inorganic CO_2 is utilised by photosynthetic organisms; oxygen is subsequently produced via photosynthesis increasing dissolved oxygen concentrations. A consecutive increase in suspended phytoplankton organic matter results in elevated turbidity levels.

4.4 Nutrient, Productivity and Hydro-period Dynamics

Chlorophyll-*a* was correlated to NO_3^--N and PO_4^{3-} across the combined dataset suggesting these key nutrients play a significant role in fuelling primary productivity. However, as illustrated in tables V.4, V.5 and V.6 relationships between nutrients and productivity displayed idiosyncratic behaviour between individual ponds.

PO_4^{3-} concentrations were positively correlated (r = 0.482) to chlorophyll-*a* in ST1, suggesting this nutrient plays a significant role governing productivity in this pond. PO_4^{3-} also correlated positively with turbidity, suggesting either an increase in phytoplankton turbidity as concentrations rise or disturbance of pond sediment during direct input events from local fauna.

Chlorophyll-*a* concentrations in ST2 displayed strong positive correlation to both PO_4^{3-} (r = 0.615) and NO_3^--N (r = 0.640). Interestingly, both nutrients showed strong correlation (r = 0.593) to each other, a relationship not observed in other ponds. This potentially suggests proportions of nutrients enter this pond from a single source, which has a significant impact of productivity. Both nutrients display strong correlation to dissolved oxygen and pH, indicating they may lead to increased photosynthetic activity. NO_3^--N were positively correlated with turbidity similar to PO_4^{3-} in ST1.

Chlorophyll-*a* in ST3 was only weakly correlated to NO_3^--N (r = 0.371). This weaker correlation observed in ST3 compared to ST2 may be due to increased productivity

from colonists, as ST3 is situated in an area dominated by *L.riparium* swards. Patches of *L.riparium* began to slowly colonise the pond in 2014 and likely compete with phytoplankton for key nutrients particularly NO_3^--N.

Spatial variations in NO_3^--N, PO_4^{3-} and chlorophyll-*a* concentrations were substantial. ST1 exhibited overall means elevated significantly above values observed in the other two ponds across the majority of the sampling period, but was particularly apparent across 2013/14. In 2013/14 NO_3^--N and PO_4^{3-} concentrations in ST1 displayed a 50.81% and 69.1% elevation from ST2, resulting in a 41.7% increase in chlorophyll-*a* concentrations. Values were similar for ST3 displaying a 66.75% and 49.74% increase in NO_3^--N and PO_4^{3-}concentrations, resulting in a 36.89% increase in chlorophyll-*a*.

In 2014/15 dynamics between the ponds were less substantial, with considerably less impact observed on productivity, particularly between ST1 and ST2. ST1 exhibited NO_3^--N and PO_4^{3-} concentrations elevated 41.21% and 50.11% above that of ST2, however, chlorophyll-*a* was enhanced by only 4.39%. Elevation above concentrations in ST3 was less considerable, displaying 11.28% and 26.69% elevation in NO_3^--N and PO_4^{3-} concentrations respectively, with only a 0.6% increase in chlorophyll-*a* concentrations. Across the 2015/16 sampling period, prior to the site flooding, NO_3^--N, PO_4^{3-} and chlorophyll-*a* concentrations were noticeably lower, more stable and varied less between ponds than in previous years (see figure V.2, V.3 and V.6).

The ponds are classified as hyper-eutrophic and display considerably higher nutrient concentrations in comparison to other freshwater systems (table V.11)**,** which would indicate these systems have reached a point of saturation. Although, these results suggest otherwise, demonstrating that further increase in nutrients, such as that observed in ST1, results in considerable enhancement of productivity. Results suggest these systems are far from saturated and have the potential for significantly more functionality.

However, in comparison to other hyper-eutrophic waterbodies nutrient concentrations and ratios of nutrients to chlorophyll-*a* in the Hauxley ponds are much higher (table V.11), yet chlorophyll-*a* concentrations are comparable, if not lower. This would suggest a factor other than NO_3^--N and PO_4^{3-} limiting rates of productivity.

Hydro-period could be a key factor limiting productivity levels within the Hauxley ponds, as dry-phases are lethal to phytoplankton and disrupt productivity as phytoplankton communities recover and re-establish upon pond rewetting. Variations in nutrient-productivity dynamics between ST1 and the other two ponds possibly reflect the different hydro-period behaviour of this pond. It was last to dry in the summer months

of 2013 and once refilled retained water when the other two ponds dried later in August. This same pattern was also observed in 2014, where ST1 retained water, whilst ST2 and ST3 underwent a ~2month long dry-phase. Extended summer wet periods in ST1, likely promoted enhanced phytoplankton growth, due to the magnification of nutrient and chlorophyll-*a* concentrations, during drawdown periods. Furthermore, as the only pond holding water it is likely that ST1 was more favourable to birds visiting the site, resulting in more localised nutrient inputs, which may account for the huge spike in PO_4^{3-} in August 2013.

Hydro-period also had another pronounced effect on nutrient-productivity dynamics. Clear pulses in increasing concentrations of NO_3-N, PO_4^{3-} and chlorophyll-*a* following pond rewetting can be observed in figures V.3 and V.6. This would indicate that nutrients are deposited in the pond via surface run-off during refill events. In some instances their also appears to be a slight lag in increasing concentrations, which indicates remobilisation of nutrients from the sediments upon rewetting, similar to processes observed in Kettle Hole ponds (Reverey et al., 2016).

Results demonstrate that nutrients are a dominant factor driving productivity within the ponds, but there also appears to be an unknown rate limiting factor, potentially attributed to pond hydro-period. Results also demonstrate that despite what would seem like saturated conditions, further elevation in nutrient concentrations can result in substantially greater productivity. This is interesting when considering the construction of ponds to provide carbon sequestration and storage services in that they clearly show the potential for ponds to be made more productive despite already hyper-eutrophic conditions. This raises an important question; can these systems be engineered and artificially enhanced to sequester and store more organic carbon?

Results reveal intrinsic relationships between hydro-period and nutrient-productivity dynamics. Retention of low water levels in summer months magnifies nutrient concentrations and enhances productivity, whilst drying leads to periods of reduced aquatic productivity, until rewetting, at which point there is a pulse in productivity and nutrient concentrations. Moreover, seasonal dry-phases also appeared to facilitate the establishment terrestrial vegetation. This was most apparent in ST2 and ST3, which began to establish patchy swards of *L.riparium* and *Agrostis*, whilst ST1 remained fairly bare, colonised largely by filamentous algae (see figure V.13).

Figure V.13 Ponds ST1 holding water and ST3 displaying the establishment of terrestrial species during dry-phase in August 2014.

4.5 Seasonal Variability and Annual Variation

Intra-annual variability of hydrology, NO_3-N, PO_4^{3-} and chlorophyll-*a* is evident in all 3 ponds (figure V.6). Concentrations are generally higher in summer and autumn, but also appear strongly affected by the hydro-period. Relationships between temperature and other physicochemical variables were similar across all 3 ponds, with temperature displaying significant correlation to all variables for the combined dataset (table V.3) and in individual ponds (tables V.5, V.6 and V.7).

Results from the PCA ordination (figure V.7) display clear separation of both seasons and years. Winter and spring were distinctly separated from summer and autumn largely aligned along the depth and chlorophyll-*a* axis. Similar seasonal separation was also observed across individual study years (figure V.8), but the degree of separation showed marked variability. Pond hydro-period was included as environmental variables in this analysis. Standing water (SW) and flooding beyond pond (FBP) were associated with winter and spring seasons. Drawdown (DD) was most closely associated with summer, whereas recharge and refill (RR) showed closer association to the autumn. Seasonally associated hydro-periods also displayed marked variation between years (figure V.8) for example drawdown periods were associated with summer in 2013/14, but were more closely associated to autumn in 2014/15.

Annual variation is also apparent with years displaying distinct separation from one another, 2013/14 were more closely related, but there is clear separation between these years and 2015/16 (figure V.7). There is also clear variability in seasonal and hydro-period, patterns and behaviour (figure V.8). Annual variations potentially reflect

climatic differences between years, which result in variable hydro-periods such as those, observed across the summer and autumn of 2013 and 2014, but may also reflect the physicochemical development of ponds as they undergo succession.

Considering the impact of hydro-period on physicochemical functioning of the ponds, it is likely that seasonal changes play an important role, but perhaps as important as climate on the whole, which is variable between seasons and across individual years, ultimately affecting the hydro-period and physicochemical functioning of the ponds.

4.6 Carbon Sequestration and Storage

All ponds exhibited high chlorophyll-*a* concentrations throughout the study period confirming disproportionate levels of productivity. Results demonstrate the enhancement of productivity in response to increased nutrient loadings despite already saturated conditions, driven by longer summer periods of standing water and less susceptibility to drying. However, in the summer of 2013 all three ponds dried to baked clay with no visual accumulation of sediment OM (figure V.14a).

Figure V.14: a) ST1 dried to baked clay in 2013. b) Deposition of algal mat and encroachment of *L.riparium* and *Agrostis* in ST3 2014. c) ST1 in 2015 displating filamentous algae and bare substrate. d) ST3 in 2015 displaying establishment of *L.riparium* and *Agrostis*.

The following summer (2014), whilst ST1 retained standing water, mats of *Spirogyra* were deposited upon the drying of ST2 and ST3 keeping the bottom of the ponds damp. Patchy swards of *L.riparium* and *Agrostis* began to establish within the ponds (figure V.14b). This had a noticeable effect on the pond bottom substrate, particularly upon refilling, with anoxic conditions apparent under degrading vegetation, the development of the moss sward creating a switch in ecosystem functioning, indicating the start of sedimentation and accumulation of OC. This may also be reflected in annual variations in the dynamics of NO_3-N, PO_4^{3-} and chlorophyll-*a* particularly in 2015/16, where observed concentrations were comparably lower than previous study years. This potentially reflects the successional development of the ponds, whereby the colonisation of vegetation results in increased utilisation and competition for nutrients.

Sediment core results (figures V.9) suggest that sediment accumulation and development is limited to the top cm of the core profile at this stage. OC storage across the 3 ponds ranged from 24.21 – 57.24 g OC m^{-2}, markedly less than values observed in the mature ponds in chapter IV. ST1 displayed an uncharacteristic sediment profile, in regards to OC%, DBD and %Moisture with the core comprising the clay bottom soil. It is likely that OC accumulation within this pond is limited and is comprised of an early semi-transitional layer between sediment and clay or that sediment accumulation was much finer than the 1 cm resolution used to dissect cores could represent. OC burial rates for new ponds were comparably lower to previous studies on mature ponds, in chapter IV and Gilbert et al. (2014), ranging from 8.07 to 8.07g OC m^{-2} yr^{-1} (table V.10).

The chemical nature of phytoplankton and plant colonists at this stage, results in deposition of highly labile OM that is easily photo-oxidised or readily degraded by microorganisms (Reverey et al., 2016). Deposition of labile OM, alongside dessication of sediments likely accounts for the rapid reminerlisation of carbon as the ponds transition from wet to dry (Gilbert et al., 2016). This would further support the theory that ponds these systems can switch from highly productive sinks of carbon during inundation, to substantial sources during drying, suggesting that their function as either a sink or source of carbon is largely driven by climate. Such dynamics have been suggested as a factor, driving the residence time of temporary aquatic habitats, such as Kettle Hole ponds, over geological time scales (Reverey et al., 2016). This climate controlled equilibrium creates substantial implications when trying to model and quantify the biogeochemical dynamics of these features, but can inform their construction as carbon mitigation tools through strategic placement and morphological design, to reduce their functionality as a source, shifting the net carbon balance to an active sink.

In the final year, vegetation in ST1 was limited to filamentous algae and the bottom substrate remaining bare (figure V.14c), whereas ST2 and ST3 displayed initial establishment of *L.riparium* (figure V.14d), which may account for the more characteristic sediment profile observed within these ponds. If a similar intensity of productivity were to be observed throughout further stages of succession, upon establishment of vegetation communities like *L.riparium* and *G.fluitans*, the characteristically more refractory OM associated with these vascular, root based species, will likely result in increased storage of OC (Reverey et al., 2016).

OC storage and burial over this stage of succession is low compared to rates observed in mature systems, despite hyper-eutrophic conditions. Results provide insights into OC burial and productivity dynamics within the ponds across early stages of succession, highlighting significant effects of nutrients, hydro-period and climate. A comprehensive understanding of these intrinsic interactions is required if we are to fully elucidate primary influential factors driving OC storage and burial in these systems. Only then can their full functionality in regards to carbon cycling be understood and implemented with confidence in landscape carbon mitigation measures.

5.0 Conclusion

Small constructed ponds are considerably more productive than other aquatic systems. High nutrient concentrations drive hypereutrophic conditions, resulting in intense levels of productivity. However, distinct variations in physicochemical functioning between ponds were apparent, driven by pond hydro-period, ultimately governed by local climate and its immediate locality. Hydro-period was crucial in driving enhanced productivity levels, during summer drawdown and upon rewetting. In terms of OC burial hydro-period also had a significant impact. Ponds retaining water through summer were more productive but stored little carbon. Ponds that dried facilitated the establishment of terrestrial plant communities, contributing to OC storage and burial, albeit at negligible rates in comparison to mature systems.

Utilising past hydrological and vegetation data from the site it becomes apparent that the same processes likely occurred within the mature ponds, some 20 years previous. Ponds in the lower part of the field, prone to dry-phases, facilitated the earlier establishment of *L.riparium*, which may account for elevated OC burial observed in these ponds (Gilbert et al., 2014). Results provide insights into the physicochemical development of small constructed ponds across early stages of succession, identifying dominant factors driving physicochemical functioning, and ultimately elucidating implications for OC burial. Results can be used to inform the construction and engineering of ponds for specific ecosystem functions, such as OC storage.

Key Findings:

1. Identify the dominant controls on primary productivity

- All 3 ponds were classified as hyper eutrophic, exhibiting concentrations of NO_3-N, PO_4^{3-} and chlorophyll-*a* well above published threshold values.
- NO_3-N and PO_4^{3-} were primary factors driving chlorophyll-*a* concentrations. Concentrations of these key nutrients were largely a avian activity in the ponds and in the surrounding area.
- Results also indicate another unknown rate limting factor on productivity, potentially associated with hydro-period.
- Hydro-period had a considerable effect on productivity. Retention of water in summer lead to enhanced productivity. Rewetting after dry-phase also resulted in a simultaneous pulse of nutrients and productivity. Dry-phases also facilitated the encroachment of more vascular vegetation.

2. Assess variations in physicochemical functioning between ponds

- The effect of local climate on pond hydrology varied considerably between years and to a lesser extent between individual ponds.
- Variations were observed in the physicochemical functioning of different ponds. Largely driven by the individual hydro-period of the ponds.
- NO_3-N and PO_4^{3-} and chlorophyll-*a* concentrations were notably lower and less variable in the final year of study prior to the whole site flooding. Potentially indicating a transition into more stable physicochemical functioning.

3. Assess the impact of initial physicochemical development on early OC burial and storage

- OC storage across the three ponds ranged from 24.21 – 57.24 g C m^2.
- OC burial rates were also comparably lower than previous studies on the mature ponds, both in chapter IV and Gilbert et al. (2014), ranging from 8.07 to 19.08 g OC m^{-2} yr^{-1}.
- OC burial and storage appeared to be limited to ponds with initial establishment of *L.riparium* and *Agrostis.*
- This suggests that despite high productivity, overall OC storage and burial over this preliminary successional stage is low in comparison to rates observed in adjacent mature systems.

Chapter VI:

Carbon Storage, Burial and Impacts of Vegetation Community Succession

Research Objectives:

1) Calculate OC storage and burial rates for the Hauxley ponds.
2) Identify significant differences in OC storage and burial between pond groups.
3) Identify the impact of past vegetation community succession on OC storage and burial.
4) Explore the implications of OC burial in a national and global context.

1.0 Introduction

This chapter builds upon a previous study, which observed OC burial rates in small constructed ponds elevated substantially above a range of terrestrial and natural aquatic environments (Gilbert et al., 2014). Considerable variation in OC storage was observed across ponds leading to uncertainty in the accuracy of coring methods, addressed in chapter IV, whilst also raising questions as to the potential effect of vegetation community succession on OC storage and burial.

Aquatic macrophytes contribute significantly to the total OC pool (TOC) of sediments in productive aquatic environments and contribute up to 60% TOC to sedimentary OM (Aichner et al., 2010). The amount of OC stored represents the balance between plant shoot and root litter production, root exudates and their microbial decomposition. Variations in OC storage have also been observed between aquatic systems displaying different vegetation communities (Dunn et al., 2015; Aichner et al., 2010). Enhanced OC storage is often associated with increased plant diversity (DeDeyn et al., 2011), reflecting either higher primary production (Marquard et al., 2009; Tilman et al., 2001), or longer persistence of plant derived OM due to slower rates of decomposition (Schmidt, 2011; Jastrow et al., 2007).

Macrophytes influence rates of OM degradation through two primary mechanisms. Firstly, is the quality and quantity of the substrate litter produced from decaying plant material, in respect to nutrient content, carbon nitrogen ratio (C:N) and composition (i.e. labile or recalcitrant) (Limpens et al., 2008; Bragazza et al., 2006). Secondly, is the quality and quantity of root exudation, part of the rhizodeposition process in plants, which involves the release of ions, inorganic acids, oxygen, water, and a broad suite of carbon based compounds (Bais et al., 2006).

In peatland systems, it is established that vegetation coverage is a significant factor determining carbon emissions and storage. *Sphagnum* species are associated with increased OC storage, whereas *Juncus* species are associated with higher rates of carbon emission. Root exudates from *Juncus* species are highly labile, creating a rizospheric priming effect (Aichner et al., 2010). Essentially this is an increase in microbial activity in response to inputs of labile OM, which is easily consumed by heterotrophic microbes. Enhanced microbial activity can facilitate the degradation of more recalcitrant OM, suggesting that *Juncus* species can play an important role in shaping OC storage within these environments. *Juncus articulatus* was one of the dominant species observed at Hauxley; *Juncus inflexus* also became established in some ponds in later years.

Limited research has been carried out on the influence of dominant vegetation type or historical vegetation community succession, on subsequent sediment OC% concentrations in ponds (Gilbert et al., 2014). The heterogenous nature of pond ecological communities is important when characterising differences in the OC burial capacity of different ponds. Exploratory studies have identified potentially significant effects of vegetation coverage on OC burial and storage. Gilbert et al., (2014) noted that hydrological driven variations in plant community succession, promoted variations in OC storage. Ponds displaying an earlier establishment of moss swards and aquatic grasses were higher in OC% and stored more OC than ponds retaining more open water flora of algae and submerged macrophytes such as stonewort (*Chara vulgaris*) or water buttercup (*Ranunculus aquatilis*).

Jeffries (2008) noted that the establishment of moss swards resulted in the bottom substrate remaining damp and anoxic, even throughout drought periods. *Chara vulgaris* and *Ranunculus aquatilis* do not create a thick blanket over bottom substrate when the ponds dry, with the substrate often drying to an exposed baked clay, revealing limited sediment development and accumulation (Gilbert et al., 2014).

Previous results from the ponds suggest a mean burial rate of 149 g OC m^{-2} yr^{-1} (Gilbert et al., 2014), which is considerable in comparison to terrestrial and natural aquatic environments (Gilbert, 2014; Boyd, 2010). Results provide further evidence to support Downing's (2010) hypothesis of the disproportionate relationship between water body size and intensity of carbon cycling and burial. The capacity of small ponds to capture large amounts of OC is realised when small semi-natural ponds accumulate OC at rates comparable to, if not elevated above, systems with a multitude of anthropogenic inputs (i.e. direct fertilisation of aquaculture ponds or indirectly from agricultural run-off). Information on species coverage and vegetation succession in relation to OC burial and storage can inform the construction of ponds as carbon mitigation features, naturally engineered through the use of specific vegetation, to provide strategic and targeted ecosystem functionality.

This chapter aims to quantify OC storage and burial across a suite of ponds at the Hauxley site, representing distinct groups of past vegetation community succession and hydrological behaviour. Data will then be compared against values published for other aquatic systems, assessing their relative effectiveness in storing and burying OC, whilst also elucidating the effects of past vegetation community in shaping sediment physicochemical conditions that promote or restrict OC storage.

2.0 Methods

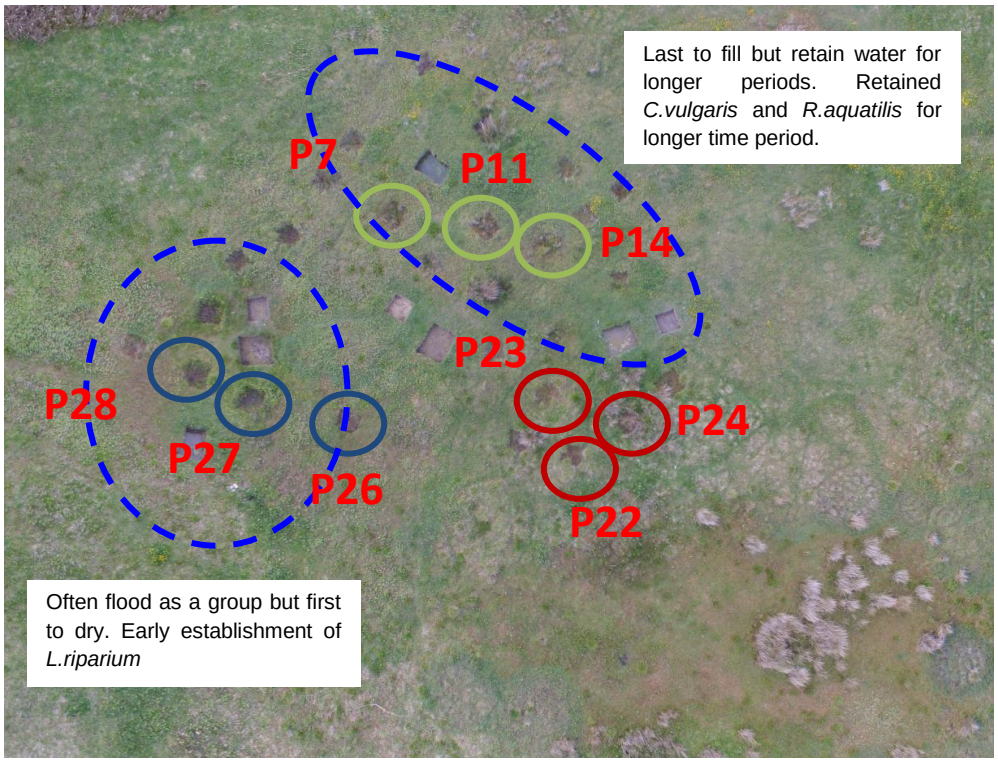
2.1 Pond Selection

Nine ponds were selected for this chapter, on the premise that they had not been cored by previous research on the site, as oxidation of the sediment layers may have resulted in losses and transformation of OC down the sediment profile.

The selection process aimed to be representative of the hydrological gradient of the site, whilst also capturing the heterogeneity observed in patterns of vegetation community succession. The site was split into three zones, largely based on the establishment of *L riparium* and a shift to more vascular plant based community (see chapter II, figure II.9) (Jeffries, 2008).

A triplicate of ponds was chosen for each zone; ponds 26, 27 and 29 (Group 3) represented ponds that displayed an early establishment of *L.riparium*, whilst ponds 7, 11 and 14 (Group 1) represented ponds that supported more aquatic based vegetation due to *L.riparium* taking longer to establish in these ponds. Ponds 22, 23 and 24 (Group 2) were included as an intermediate set of ponds along the pathway of *L.riparium* establishment, but also represent displaying earlier establishment of *J.articulatus*. Ponds selected can be seen in figure VI.1 and II.13.

Fig VI.1: Satellite image highlighting sampled ponds. Green Circled ponds (Group 1: 7, 11, 14) Blue circled ponds (Group 3: 26, 27, 28 Red circled ponds (Group 2: 22, 23, 24).



2.2 Sediment Cores

Sediment cores were extracted using the newly developed stainless steel 4.7 cm diameter corer (see chapter III). Cores were extruded and separated at 1 cm intervals within the field and placed in pre-weighed tin foil and sample bags. Samples were then returned to the lab where they were weighed and placed to air dry for ~7 days at 60°C. Samples were then weighed to obtain dry weights prior to grinding and sieving at 500 µm.

2.3 Elemental Analysis

EA was used to analyse OC and Nitrogen concentrations. Samples were analysed on a Thermo Scientific Flash 2000 Organic Elemental Analyser configured to determine CN.

2.4 XRF

Analysis for aluminium, iron, magnesium, phosphorus, potassium and sulphur was conducted using XRF. Samples were first placed in a ball mill for 180 seconds before being made into pellets. Pellets made up of ~4 g of sample and ~0.7 g of FluXana CEREOX Licowax binder. Samples were then analysed via a Spectro X-lab 2000 and Spectro XEPOS .

2.5 Sediment Depth Layer determination

Detrended Correspondance Analysis (DCA) was used based on results in chapter IV, to determine the depth layer of the accumulated sediment-clay interface in order to produce accurate estimations of OC storage.

2.6 Sediment Core Carbon storage estimations

Sediment Core Extrapolation to whole pond calculation:

i) Carbon Stock in individual sediment layer = C Density (mg OC cm^{-3}) x Volume of sediment layer (cm^3)

ii) Extrapolation to whole pond = Sum of carbon stock in accumulated sediment layers identified by DCA (g OC) / Surface area of corer (cm^2) x 10000 (Dimensions of $1m^2$ Pond)

Burial rate calculation

Carbon Burial Rate = Carbon Stored (g OC) / Age of pond (yrs)

2.7 Statistical Analysis

2.7.1 ANOVA repeated measures

An ANOVA model including depth as a repeat measure was used to establish significant differences in sediment physicochemistry between pond groups. ANOVA was performed in SPSS statistical software package.

All variables apart from C density did not follow normal distribution, so were log10 transformed to improve normality. A post hoc Bonferonni test was applied to establish where significant differences were between the pond groups.

2.7.2 Spearmans rank correlation

Past vegetation community succession data was transformed in three stages. Initially, the mean coverage for individual species was established for each pond, this was then converted to % of the maximum mean observed through the studied ponds. Given that this data is represented as proportional percentage data, results were arcsine transformed prior to Spearmans rank correlation analysis with OC storage values. Regression plots for the vegetation data against OC storage also utilised this arcsine transformed data.

3.0 Results

3.1 OC%, DBD AND C Density

Sediment OC% in cores from the site survey ranged from 1.46% – 37.83% (n=135) and varied considerably between both ponds and down the core profile (figure VI. 2a)**.** OC% was generally highest and expressed more variation between ponds, in the upper sediment layers to depths of around 5-8 cm, after which levels become fairly constant and stable with depth.

DBD also varied substantially between ponds and down the core profile ranging from 0.054 – 2.75 g cm^{-3} (figure VI.2b). DBD was highest throughout the lower depth layers reflecting differences in the physical composition of accumulated sediment and underlying clay soil.

C density also appears elevated in the upper layers of sediment particularly across ponds 26, 27 and 28 (Group 3) (figure VI.2c). Values across all ponds ranged from 10.03 – 86.22 mg OC cm^{-3}.The substantial decrease in OC% with depth appears somewhat negligible in overall C density once DBD is factored in.

3.2 XRF Analysis

XRF analysis was performed on sediment cores to determine whether down core trends observed across exhumed pond cores in chapter IV were observed in these cores. Results for organic variables N (EA analysis), P and S can be observed in figure VI.3. Results for clay soil variables can be seen in figure VI.4. Results confirm similar down core trends in aforementioned variables, which support their use in the multivariate analysis determination of the sediment-clay interface.

3.3 Sediment-Clay Interface Determination

DCA was performed on individual ponds in order to determine the sediment-clay interface layer. When aiming to determine this visually, it was found that there was an element of subjectivity given the transitional nature of the sediment into heavily rooted bottom soil. Percentage variance explained by the DCA was high ranging from 88.3 – 97.6%. Ordination plots can be seen in figures VI.5, VI. 6 and VI.7; highlighted sample points indicate samples determined to be accumulated sediment layers, which will be used to estimate overall OC storage. Table VI.1 highlights the depth layer determined for sediment layers in individual ponds and their associated OC storage estimate.

Figure VI.2: Sediment core physicochemical variables

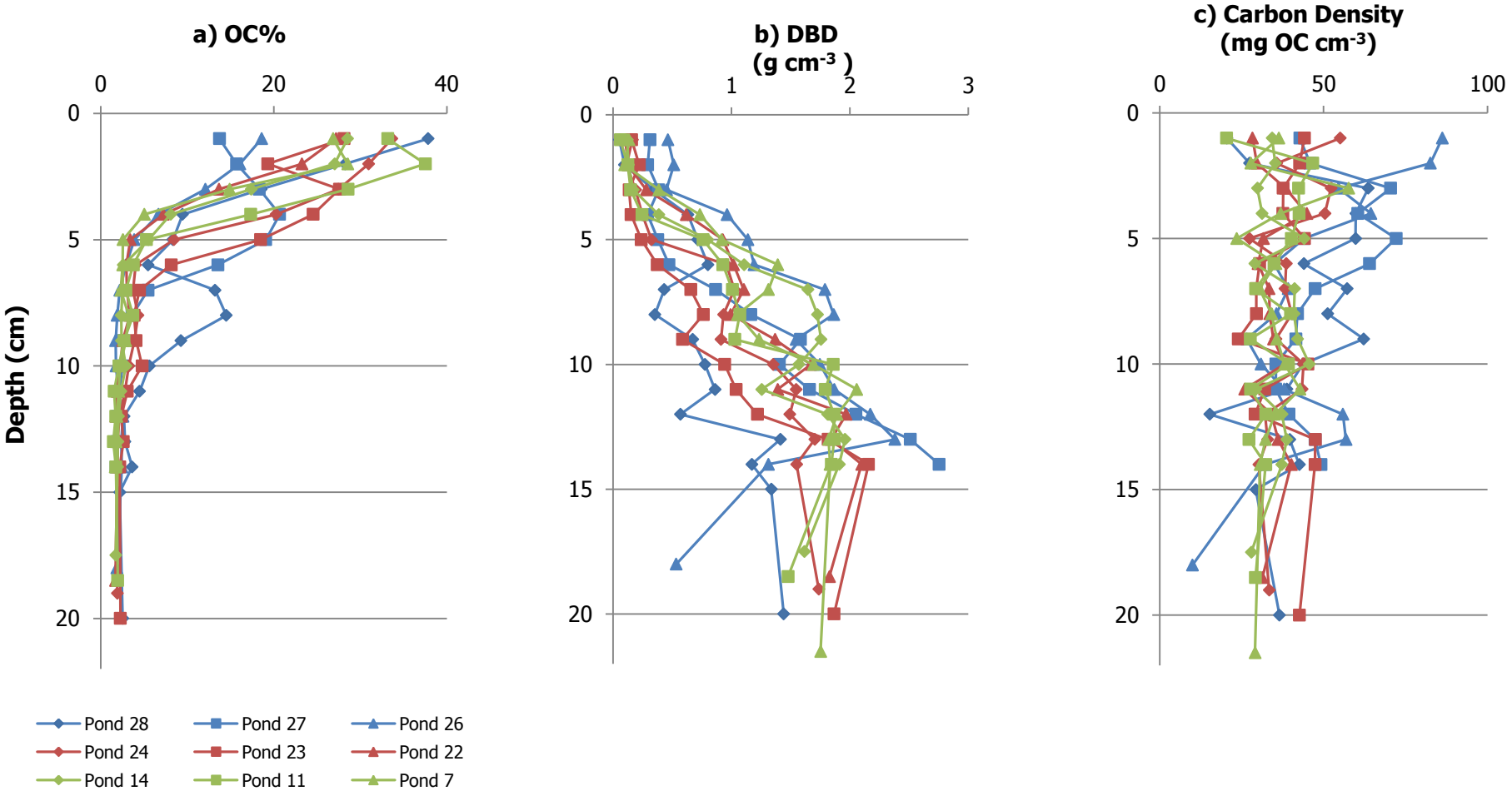


Figure VI.3: Sediment core physicochemistry – sediment layer variables.

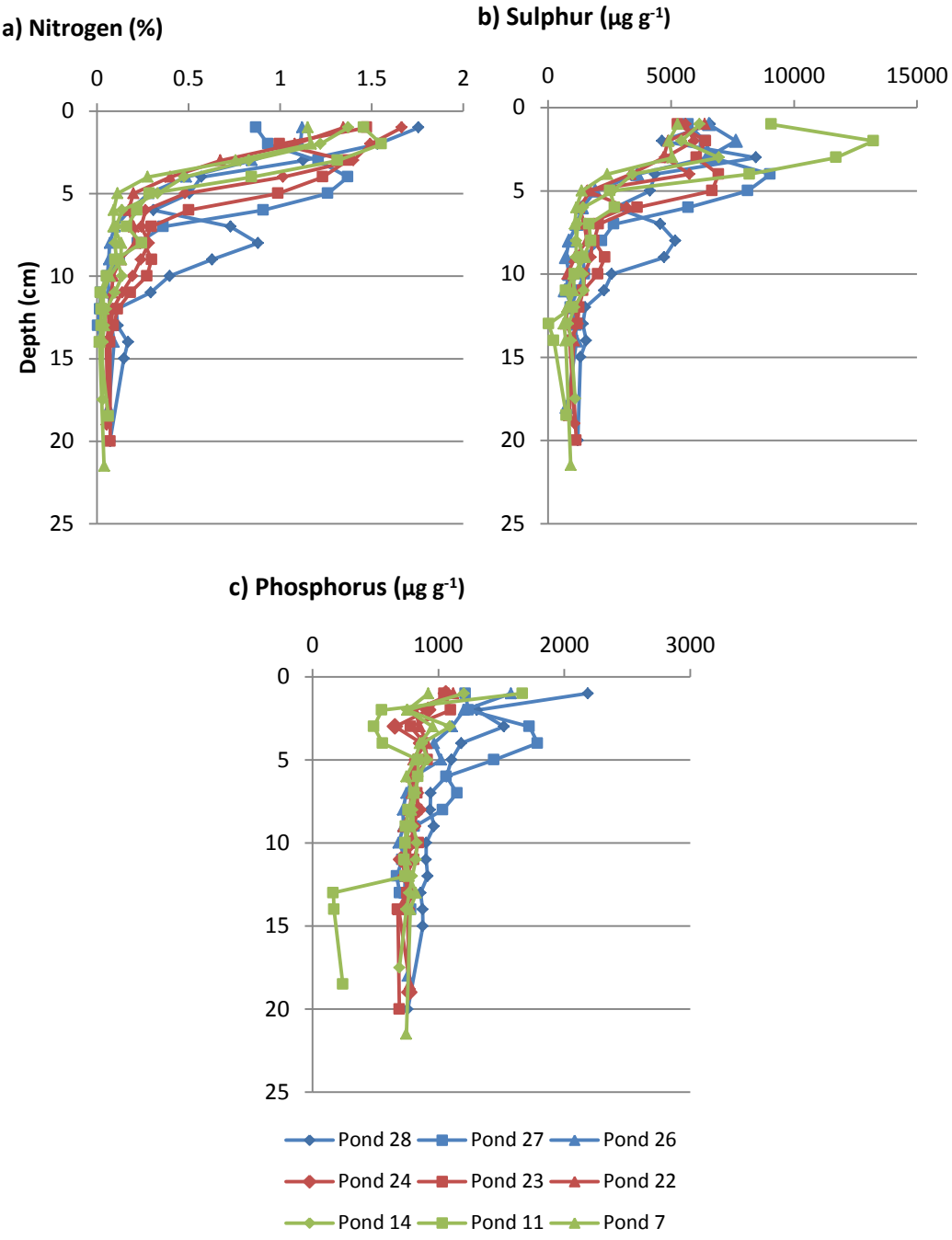


Figure VI.4: Sediment core physicochemistry - clay soil variables.

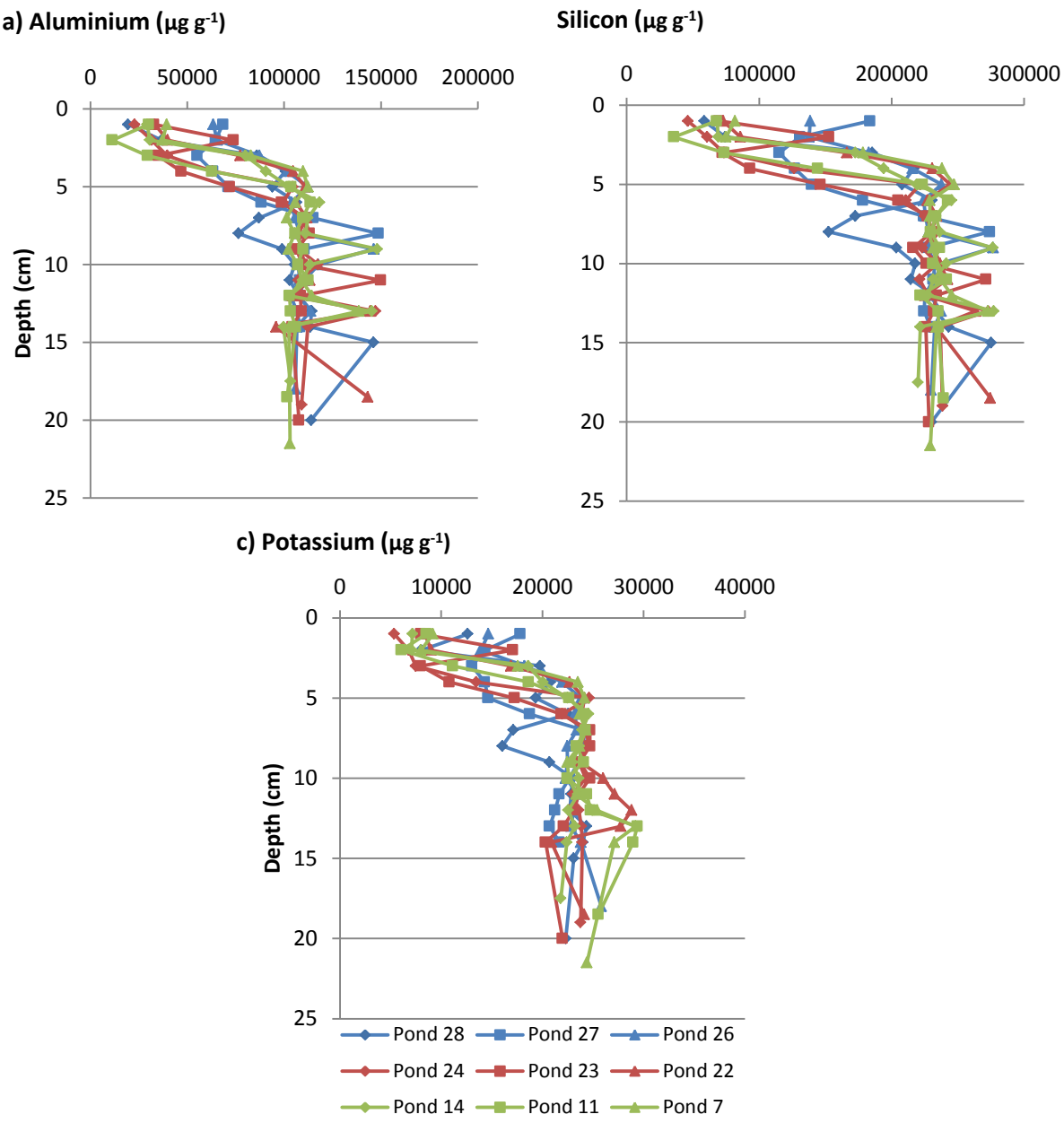


Figure VI.5: DCA analysis Group 1 pond cores. Highlighted points indicate sediment layers.

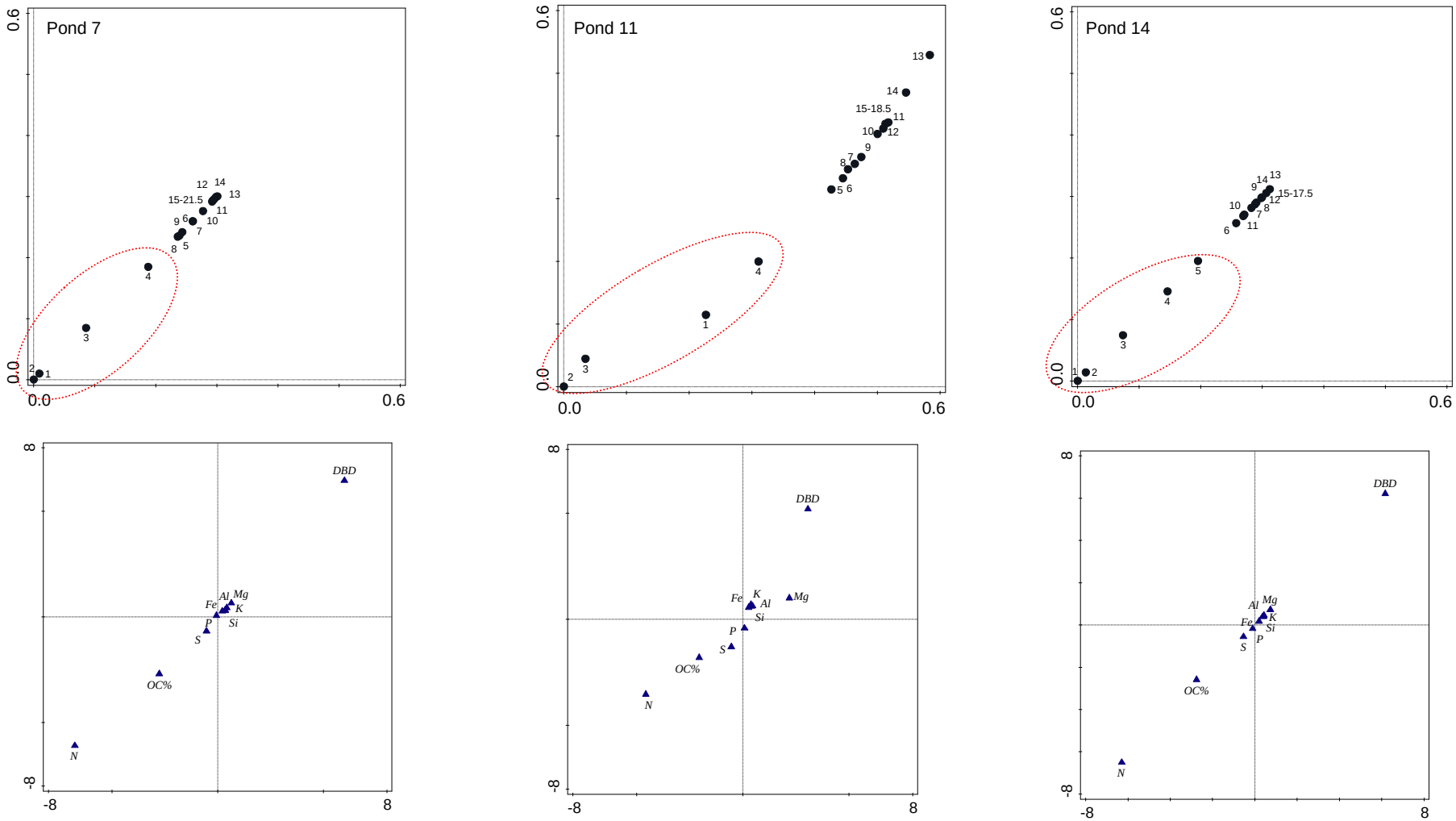


Figure VI.6: DCA analysis Group 2 pond cores. Highlighted points indicate sediment layers.

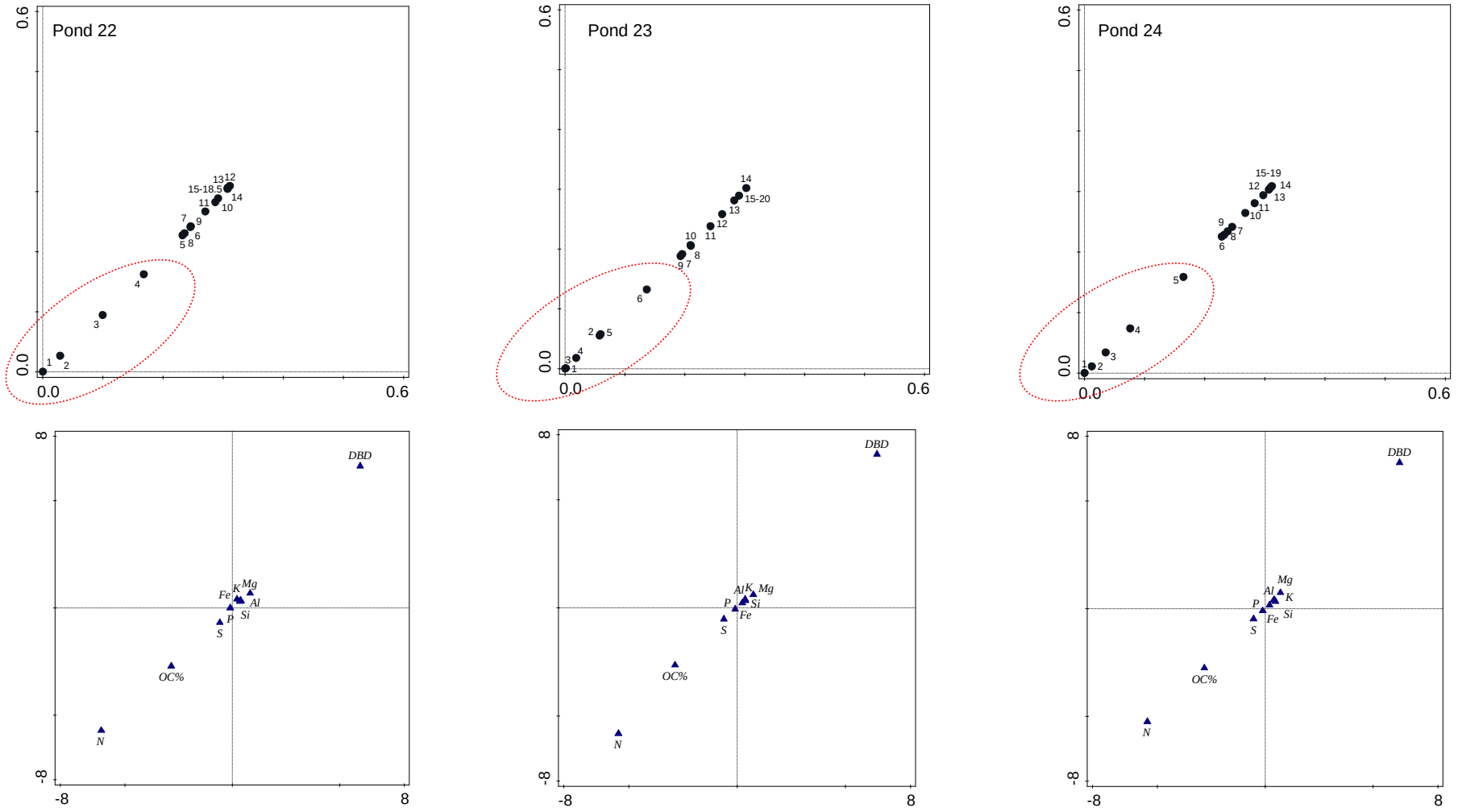


Figure VI.7: DCA analysis Group 3 pond cores. Highlighted points indicate sediment layers.

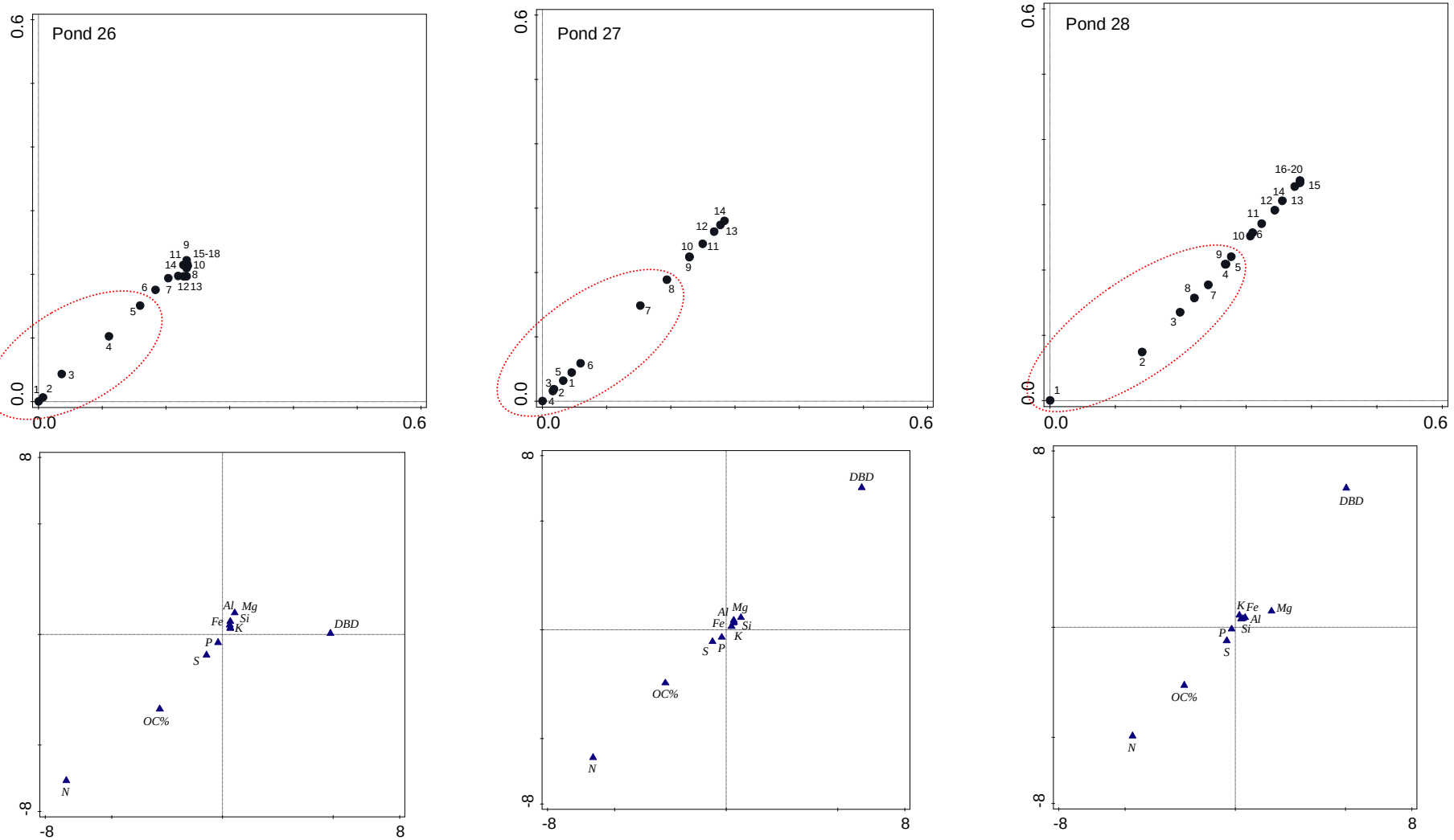


Table VI.1: Individual ponds depth layer determined by DCA and associated OC storage value.

Pond ID	Depth Layer Determined (cm)	C Storage Estimate (g OC m^{-2})
P7	4	1587
P11	4	1521
P14	5	1747
P22	4	1413
P23	6	2368
P24	5	2208
P26	5	3320
P27	8	4453
P28	9	4459

3.4 Carbon Storage and Burial

Figure VI.8 highlights estimated OC storage values for individual ponds and associated error based on the sediment core accuracy determined in chapter IV. Estimated OC storage values for sampled ponds ranged from 1413 ± 184.96 to 4459 ± 583.68 g in a whole pond with an overall site average of 2564 ± 335.63 g.

Variations in OC storage between different pond groups were similar to those observed between exhumed ponds in chapter VI. Ponds in group 3 displayed elevated OC storage values above group 1 ponds. Ponds in group 2, the intermediate area of the site displayed values somewhat in between those observed for the top and lower end of the site, apart from pond 22 which was the lowest value observed across the study.

Figure VI.8: Graph showing OC storage for sampled ponds. Errors utilised from core accuracy determined in chapter IV

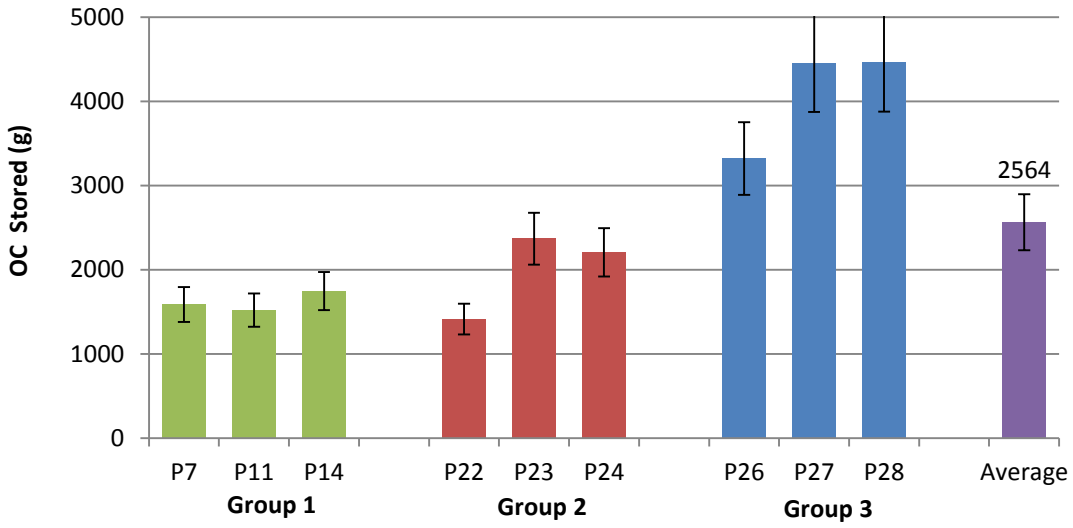
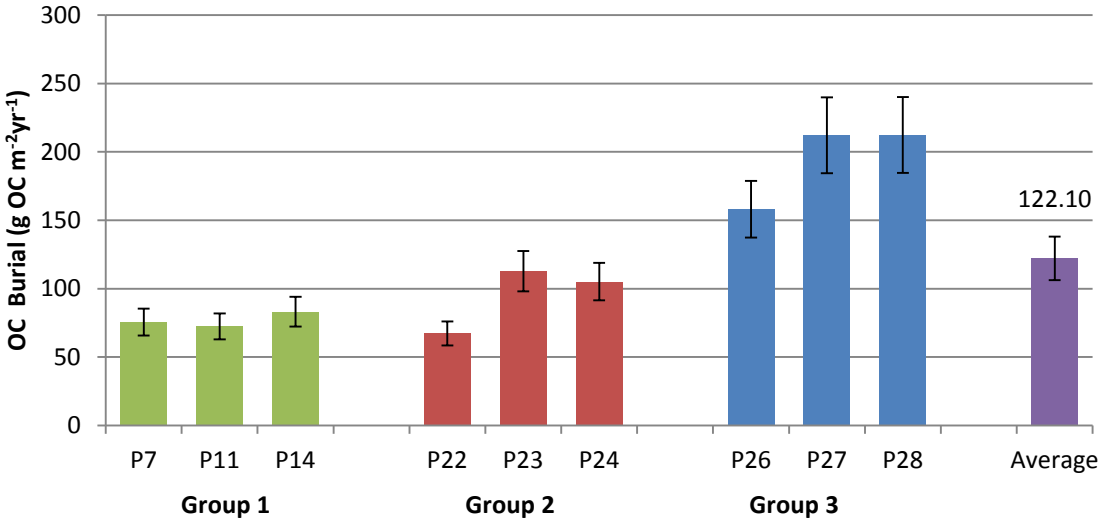


Figure VI.9 highlights OC burial rates observed across the sampled ponds, calculated by dividing OC storage estimates by the known age of the ponds. OC burial rates ranged from 67 ± 8.77 g OC m^{-2} yr^{-1} to 212 ± 27.75 g OC m^{-2} yr^{-1} with an average of 122.10 ± 15.98 g OC m^{-2} yr^{-1} for single cores taken as part of the site survey.

Figure VI.9: Graph showing OC burial rates for sampled ponds.



3.5 Variations between Ponds - Repeated Measures ANOVA

ANOVA analysis was conducted on all physicochemical variables measured in the sediment cores. Results of the analysis can be seen in table VI.2. For the majority of variables no statistical significance was observed between different pond groups. However, statistical significance was observed in C density between groups 1-3 and for phosphorus concentrations between groups 2-3.

3.6 Impact of Vegetation Community Succession

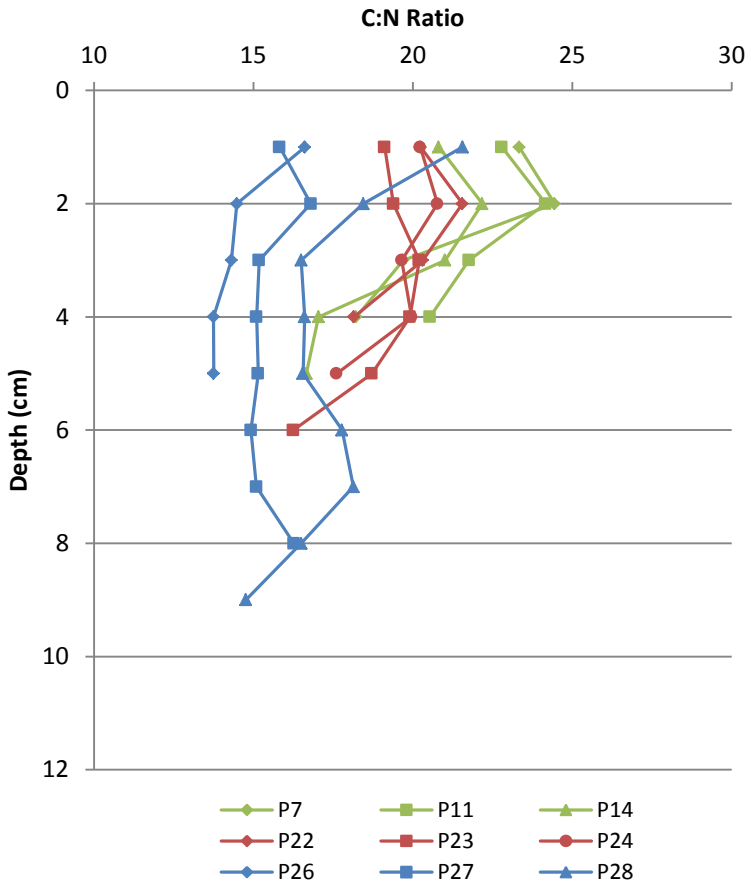
3.6.1 C:N Ratios

C:N ratios showed a general decrease down the sediment profile across the ponds (figure VI.10) and ranged from 13.75:1 - 24.45:1. C:N ratios also displayed marked separation between pond groups, yet did not display statistical significance on the ANOVA analysis. Means for each of the pond groups were 21.1:1, 19.5:1 and 15.9:1 for groups 1, 2 and 3 respectively, indicating that vegetation community succession has influenced sediment C:N ratios.

Table VI.2: Repeated measure ANOVA results with pair wise comparisons displayed between pond groups.

	Measured Variable							
	DBD	OC%	C Density	Nitrogen	Phosphorus	Sulphur	CN Ratio	Moisture Content
Grouping Category 1=ponds with more open aquatic flora, late in developing extensive *L.riparium* communities 2=Intermediate Group 3=Ponds displaying much earlier establishment of *L.riparium* and vascular based species	F = 0.161 DF 2, 46.914 P = 0.852 Pairwise p-value 1-2 = 1.000 1-3 = 1.000 2-3 = 1.000	F = 0.067 DF 2, 13.373 P = 0.935 Pairwise p-value 1-2 = 1.000 1-3 = 1.000 2-3 = 1.000	F = 4.238 DF 2, 22.966 P = 0.027 Pairwise p-value 1-2 = 1.000 **1-3 = 0.029** 2-3 = 0.165	F = 0.218 DF 2, 12.904 P = 0.807 Pairwise p-value 1-2 = 1.000 1-3 = 1.000 2-3 = 1.000	F = 4.768 DF 2, 24.920 P = 0.018 Pairwise p-value 1-2 = 1.000 1-3 = 0.070 **2-3 = 0.024**	F = 0.229 DF 2, 20.114 P = 0.798 Pairwise p-value 1-2 = 1.000 1-3 = 1.000 2-3 = 1.000	F = 1.209 DF 2, 15.554 P = 0.325 Pairwise p-value 1-2 = 0.478 1-3 = 0.797 2-3 = 1.000	F = 1.883 DF 2, 14.910 P = 0.187 Pairwise p-value 1-2 = 1.000 1-3 = 0.556 2-3 = 0.241

Figure VI.10: Graph showing C:N ratios down the core profile for individual ponds.



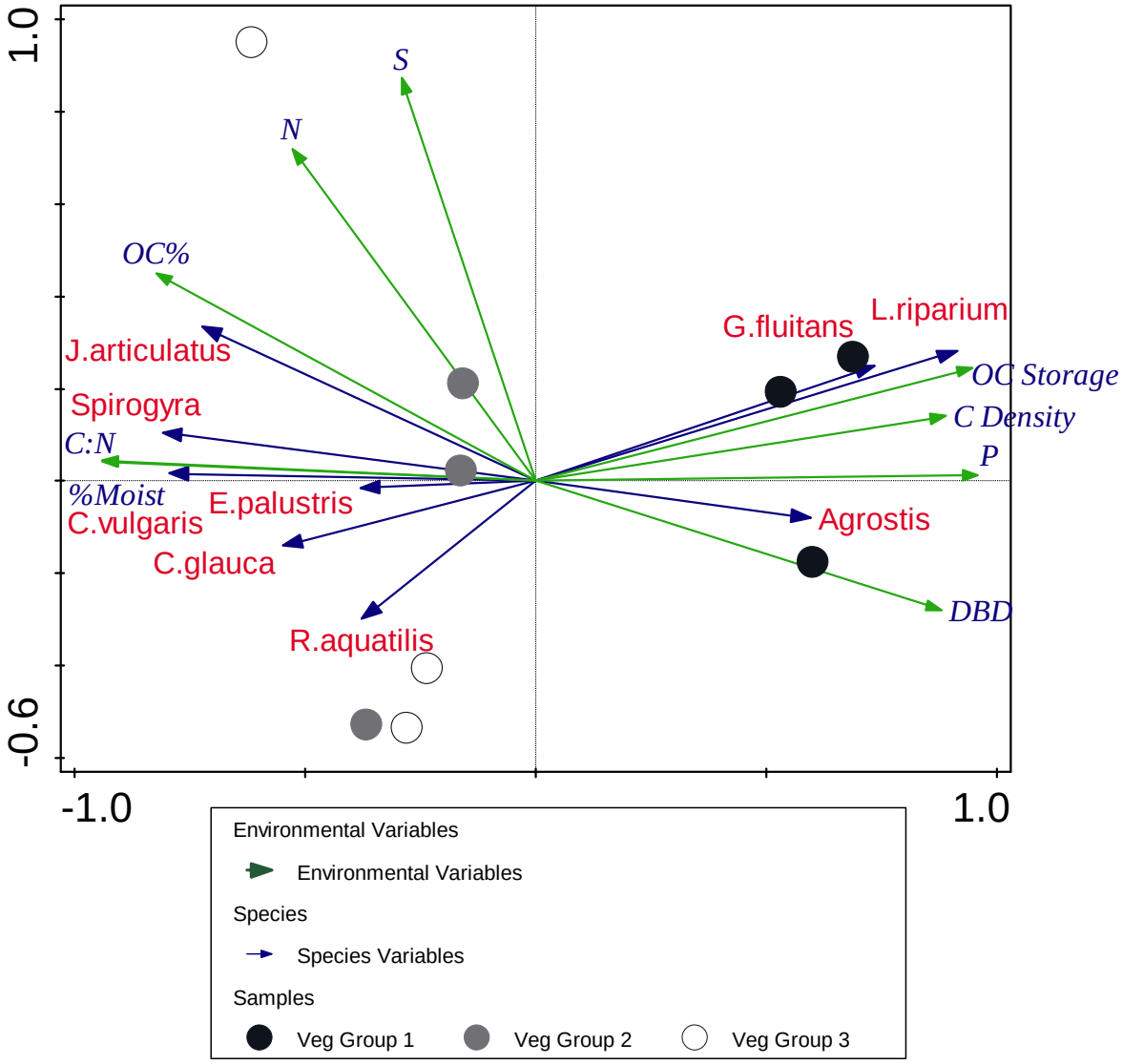
3.6.2 Principal Component Analysis

Principal component analysis (PCA) was used to assess relationships between particular vegetation species, sediment physicochemistry and OC storage. Figure VI.11 displays a PCA ordination (cumulative %variance by two axis 90.12) to separate samples (individual ponds) based on vegetation (environmental variables) and physicochemical data (species variables).

Results show clear separation between pond groups. The horizontal axis separates ponds based on OC%, %moisture and C:N, against DBD, C density and OC storage.

Vegetation aligned along the axis separate aquatic based communities of *C.vulgaris*, *Spirogyra and J.articulatus* to more vascular and emergent species such as *Agrostis*, *L.riparium* and *G.fluitans*. *E.palustris*, *C.glauca* and *R.aquatilis* showed less distinct separation along the horizontal axis, although *C.glauca* and *R.aquatilis* were more closely aligned with aquatic groups.

Figure VI.11: PCA ordination displaying physicochemical variables overlain by unconstrained vegetation coverage data as environmental variables.



Pond groups display distinct separation. Most notably this is the separation of ponds in Group 3 (26, 27, and 28) associated with *G.fluitans* and *L riparium*, from other ponds in Groups 1 (7, 11, 14) and 2 (22, 23, 24). General separation between the other two groups of ponds is also apparent, albeit much less considerable than that with Group 3.

Some vegetation communities showed close association with physicochemical variables. These include OC% with *J.articulatus*, phosphorus with *Agrostis*, and both % moisture content and C:N ratio with *C.vulgaris* and *Spirogyra*. To a lesser extent C density and OC storage were more closely associated with *L.riparium* and *G.fluitans.*

3.6.3 Spearmans rank correlation analysis

Spearmans rank correlation analysis was used to identify significant relationships between vegetation coverage and OC storage. Table VI.3 displays Spearman's correlation coefficient and p-values for vegetation coverage of dominant species observed across the site over the 20 years with OC storage.

Table VI.3: Spearman's correlation coefficient and significance values obtained for vegetation species and OC storage.

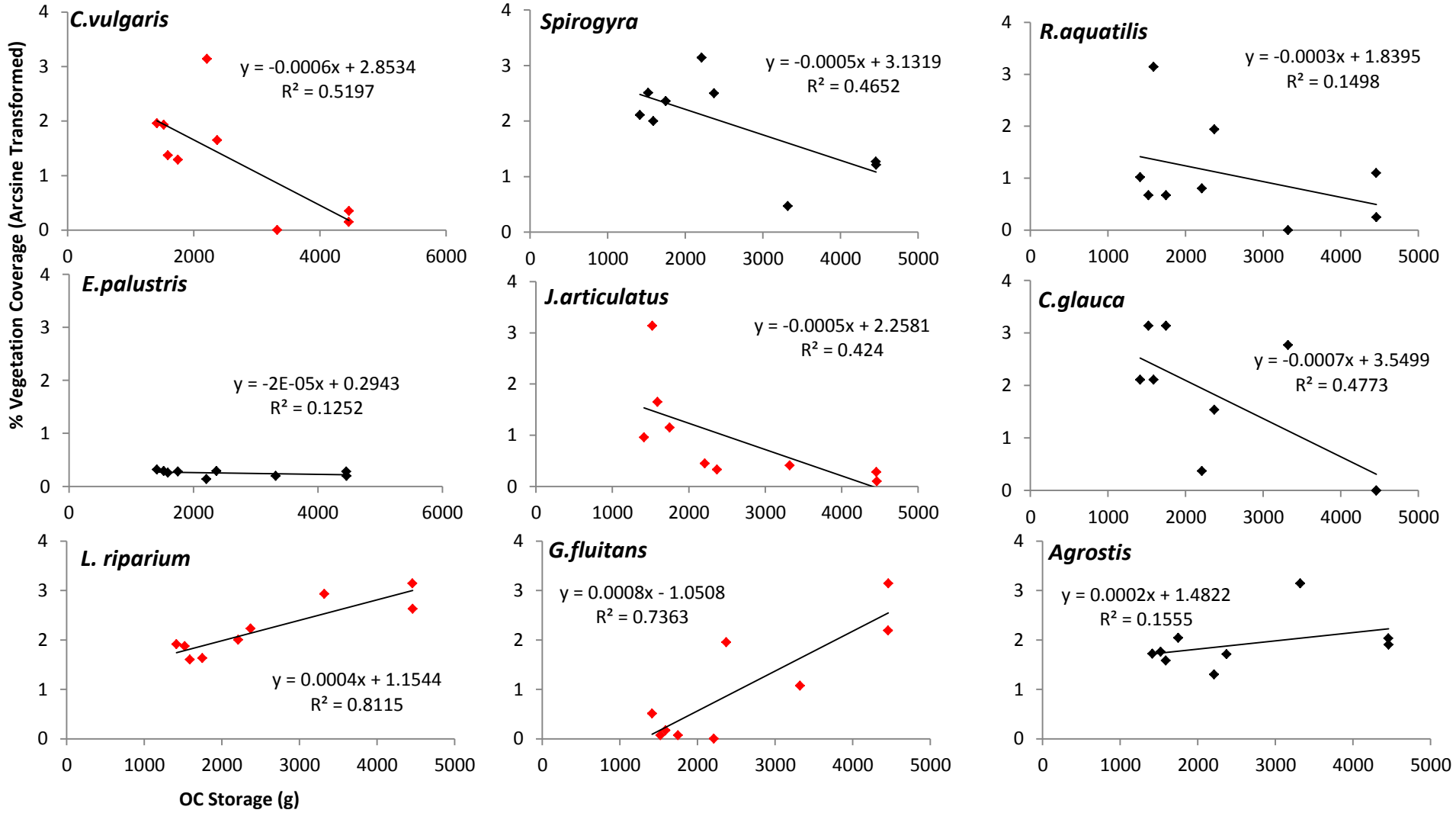
Species	Correlation Coefficient	p-value
Chara vulgaris	-0.683	0.042
Spirogyra	-0.533	0.139
Carex glauca	-0.650	0.058
Juncus articulatus	-0.883	0.002
Eleocharis palustris	-0.600	0.088
Ranunculus aquatilis	-0.285	0.458
Glyceria fluitans	0.686	0.041
Leptodicytum riparium	0.800	0.010
Agrostis	0.383	0.308

Vegetation groups that displayed statistical significance ($p<0.05$) with OC storage were *C.vulgaris*, *J.articulatus*, *G.fluitans* and *L.riparium*. *C.vulgaris* and *J.articulatus* were negatively correlated; *G.fluitans* and *L.riparium* were positively correlated.

Regression analysis was conducted to measure the degree of explanation of vegetation community succession on OC storage. Analysis was performed on all species but of most significance were those displaying p-values <0.05 in the previous correlation analysis. Regression equations and coefficient values are displayed alongside fitted line plots in figure VI.12. Negative regression with OC storage was observed for *C.vulgaris* and *J.articulatus*, displaying R^2 values of 0.52 and 0.42 respectively. *L.riparium* and *G.fluitans* displayed positive and much stronger regression, with respective R^2 values of 0.81 and 0.74

Alongside figure VI.11, results suggest that vegetation community succession plays a significant role in shaping sediment physicochemistry. Vegetation succession was largely determined by hydrology. It is likely that a combination of hydrology and vegetation create distinct environmental conditions, driving variations observed in sediment physicochemistry between ponds, ultimately affecting OC storage and burial.

Figure VI.12: Fitted line plots displaying regression equations for past vegetation coverage (% of maximum mean observed, plotted as standard residual) against carbon storage. Points highlighted in red indicate species displaying statistical significance with OC storage.



4.0 Discussion

4.1 Sediment Cores

High OC% in the upper sediment layers is typical of surface sediment layers characterised by Munsiri (1995) with low DBD and high %moisture. The trend of increasing DBD with depth is also well documented (Munsiri et al., 1995; Dadey et al., 1992). It has been speculated that such conditions slow rates of OM degradation, preserving OC to a greater depth (Gilbert et al., 2014). Despite higher OC% in upper layers, overall C density remains relatively stable with increasing depth, as increasing DBD factors against the drop in OC%. However, C density in the upper layers is elevated in the majority of cores across the site.

4.2 Sediment-Clay Interface Determination

The sediment-clay interface was determined through DCA analysis, using the same parameters and transformation as the exhumed pond cores (see chapter IV) see figures VI.5, VI.6, VI.77 and table VI.1. However, in one instance this was difficult to distinguish, with the pond 28 core displaying a recession of points in lower layers of the core back along the OC% and N axis. In other cores there were often a cluster of points, roughly across the 6 - 9cm depth layers, prior to points grouped around distinct clay variables. This is likely the result of rooted clay soil layers exhibiting a higher OC% content and slightly decreased DBD in relation to underlying clay layers, as this often occurs for just 2-3 sample points prior to a shift back along the DBD axis.

Accumulated sediment was assumed to be inclusive of all samples prior to the sample point it was determined the clay soil layer had been reached. Including rooted and clay layers in OC storage estimates would result in substantial overestimation of OC storage given the higher DBD of these samples, which would assume the ponds have accumulated a large amount of sediment mass, which in reality would be the underlying clay soil in which the ponds were constructed. It is felt that the points selected would provide conservative estimates of OC storage.

Interestingly this pattern was not observed in the exhumed pond cores (see chapter IV) and may be a result of the slightly higher sampling resolution of lower depth layers, which has the ability to distinguish between inherent differences down the core profile, given the greater representation of clay samples in the analysis. Alternatively, because the sediment cores for this chapter were taken during a dry-phase, which may result in increased root density beneath the sediment-clay interface as vegetation responds to changes in moisture availability.

4.3 Carbon Storage Estimates

Estimates of OC storage ranged from 1413 ±184.96 to 4459 ± 583.68 g OC m^{-2} with an overall pond average of 2564 ± 335.62 g OC m^{-2}. Variations in OC storage between pond groups are also highlighted in figure VI.8. Ponds in group 3 displayed higher OC storage, elevated considerably above those observed in the other two groups. Ponds in group 2 were on average slightly higher than group 1, although the variation is not as considerable as that observed for group 3 ponds. This likely arises from the significant difference observed in C density between groups 1 and 3 (table VI.2).

If this is extrapolated to cover the 30 small ponds on the site, then collectively they are storing around 76,920 ± 10,069 g or 76.92 ± 10 Kgs of carbon and have accumulated this over a period of 20 years from when they were first constructed. Under the wider context of climate change, results from overall OC storage in the experimental ponds may seem insignificant, as they are limited in their ability to store vast quantities of OC due to their small size. However, more interestingly and perhaps importantly, is the rate at which these systems have captured this amount of carbon and the implications this has under a broader landscape picture.

4.4 Variations Between pond Groups

Results from the repeated measures ANOVA analysis (table VI.2) suggest that for the majority of physicochemical variables, no statistical significance is observed between ponds, perhaps a result of relatively low replication within pond groups. However, statistical significance was observed in C density between groups 1-3 and phosphorus concentrations between groups 2-3. These results are surprising given the fairly substantial variations observed in overall OC storage between pond groups, which would indicate that there will also be significant differences in sediment physicochemistry.

Results suggest that potentially significant factors affecting OC storage are concentrations of phosphorus within the sediment and C density. Significant differences in phosphorus concentrations between the ponds may be indicative of increased external loading or an increased sorption capacity of the sediment, both of which are perhaps governed by past vegetation community succession. Group 3 ponds were recorded as regularly flooding as a group, which may also account for increased phosphorus concentrations and physicochemical differences within the sediment due to loadings from the surrounding terrestrial area in these flood events.

Of most significance is C density, which displayed significant difference between the pond groups 1 and 3, despite neither OC% nor DBD (variables used to calculate C density) displaying statistical significance. Given that these groups were selected as having contrasting past vegetation community succession, this is likely a significant contributory factor. Group 2 was included as an intermediate group in terms of vegetation succession development between aquatic and terrestrial species. This may account for why no statistical significance was observed in C density between groups 1 and 2, or 2 and 3. C density is a substantial factor in calculating overall OC storage, factors affecting the C density are crucial to elucidating the effectiveness of constructed ponds at capturing and storing OC.

4.5 Impacts of Vegetation Community Succession

C:N ratios (figure VI.10) generally decrease down the core profile and vary considerably between ponds despite no significant statistical difference being observed. Algae have a C:N ratio between 4 and 10, whilst terrestrial OM has a C:N greater than 20 (Kauschal & Binford, 1999; Meyers, 1994). Values observed in this study ranged from 13.75:1 - 24.45:1, suggesting contribution of both terrestrial and algal based OM to the sediment. Decreases in C:N ratios down the core profile have been associated with a higher proportion of algal OM whilst an increase suggests higher proportional inputs of terrestrial OM (Kauschal & Binford, 1999; Guilizzoni et al., 1996; Kanassanen & Jaakkola, 1985). The general decrease down the core profile observed across most ponds likely reflects a higher proportion of algal OC entering the system in earlier years (1994-1999) prior to the establishment of species such as *L.riparium*, *Agrostis* and *G. fluitans*.

C:N ratios displayed distinct differences between pond groups. A mean of 21.1, 19.5 and 15.9 observed for groups 1, 2 and 3 respectively. Interestingly higher C:N ratios were observed within the group 1 ponds which had retained a more open flora of *Spirogyra* and submerged aquatic plant species which would not necessarily agree with the literature. However, the validity of using C:N ratios to determine changes in OM source has been questioned (Kauschal & Binford 1999), largely due to diagenetic alterations observed in C:N ratios, which decreases C:N values for terrestrial OM whilst increasing values for aquatic OM.

It is possible that such diagenetic alterations have resulted in the variation observed between ponds. Proportions of terrestrial OC inputs are higher in the group 1 ponds despite displaying greater coverage of aquatic species throughout their development. This may arise due to the labile nature of aquatic based OM which results in

preferential degradation of these sources, which increase the C:N ratio through diagenetic alteration and lead to a higher overall contribution of terrestrial OM as labile aquatic OM is remineralised and lost from the sediment. These ponds stored the least OC, indicating higher rates of degradation. Decreased C:N ratios in group 3 could potentially be driven by diagenetic alterations of terrestrial OM increasing the C:N ratio, but could also indicate higher preservation rates of aquatic OM.

Alongside results from the PCA analysis (figure VI.11), it is clear vegetation community succession has a significant impact on OC storage. Ponds in group 3, that displayed earlier development of *L.riparium*, Agrostis and *G.fluitans,* were denser and stored more OC. This may also account for the significant difference in C density observed between group 1. Ponds that retained aquatic based flora of *Spirogyra*, *C.vulgaris*, *R.aquatilis*, later dominated by *J.articulatus* were wetter and more OC rich, but stored comparably less OC. Results suggest vegetation community succession plays a significant role in the physiochemical development of pond sediment.

Correlation analysis identified species displaying significant relationships with OC storage (see table VI.3). Coverage of *L.riparium* and *G.fluitans* was positively correlated to OC storage, whilst coverage of *C.vulgaris* and *J.articulatus* displayed negative correlation. These species were the most widespread across the site, the establishment of *L.riparium* communities often coinciding with the loss of *Spirogyra* and *C.vulgaris*. Significant correlation observed with OC storage indicates that earlier establishment of *L.riparium* and *G.fluitans* played a significant role in promoting OC storage. Negative correlation observed with *C.vulgaris* and J*.articulatus* indicates that these species reduce OC storage capacity. *J.articulatus* could negatively affect OC storage through “rhizospheric priming effect”. Peatlands dominated by *Juncus* species store less OC and have higher CO_2 emission rates. It is understood that highly labile root exudates from *Juncus* species, also evident in association of *J.articulatus* with OC% (figure VI.11), trigger an increase in microbial activity, increasing OM degradation and facilitating the breakdown of more recalcitrant OM (Lange et al., 2015).

It was documented that the establishment of *L.riparium* communities caused distinct physical changes in the sediment, particularly throughout drought periods, keeping sediment damp and anoxic (Gilbert et al., 2014; Jeffries, 2008). Similar patterns were also observed in chapter V. Aquatic plants are often composed of lower to mid-chain structural components such as n-alkanes and are more prone to degradation than terrestrial and emergent species such as *L.riparium* and *G.fluitans* which are composed of longer chain and molecularly heavier structures (Reverey et al., 2016; Lange et al., 2015; Aichner et al., 2010).

The exposure of labile aquatic OM in drought events likely resulted in the degradation of the majority of this OM, restricting sediment accumulation. Aichner et al. (2010) suggests that in drought conditions some species of emergent macrophytes biosynthesise molecularly heavier compounds, more resistant to degradation. This could also be a factor driving OC storage in the group 3 ponds, which are more susceptible earlier dry-phase in warmer months.

Coverage of *L.riparium* across the bottom substrate limits evaporation of moisture during drought events, which provides an environment for *L.riparium* to grow without competition from aquatic species. Physical changes to the sediment, notably the retention of moisture and formation of initial sediment layers, facilitate the establishment of emergent root based species such as *J.articulatus*, *Elecocharis palustris* and *Glyceria fluitans* resulting in the loss of strictly aquatic flora and establishment of plant species with more refractory OM.

This is a complex interplay between hydro-period, vegetation succession and physicochemical development of sediment layers that ultimately affect OC burial.

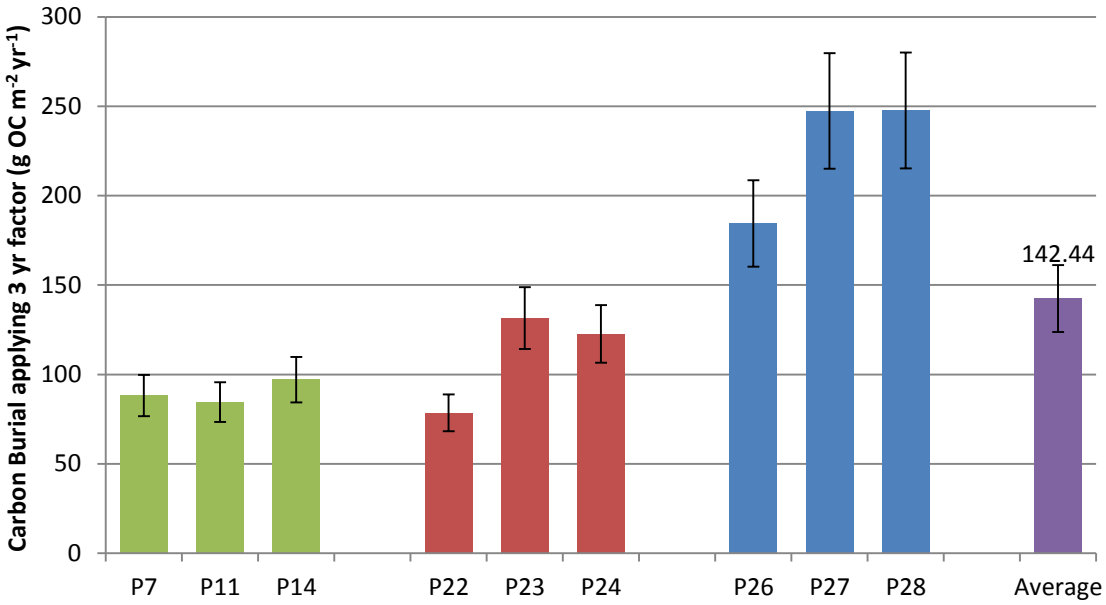
4.6 Carbon Burial

The known construction age of the ponds coupled with the ability to accurately estimate the amount of OC stored, allows OC burial rates to be calculated. Burial rates observed across the ponds can be viewed in figure VI.8. Rates ranged from 67 ± 8.77 to 212 ± 27.75 g OC m^{-2} yr^{-1} with an average of 122.10 ± 15.98 g C m^{-2} yr^{-1}.

However, it is likely that OC burial is not uniform throughout the development of the ponds. Initial stages of succession, where vegetation communities were limited to *Spirogyra* and pioneer species such as *C.vulgaris,* often resulted in ponds drying to an exposed baked substrate during summer drought periods. Results from chapter V confirm this, indicating that throughout the first three years of development, rates of OC burial are negligible (8.07 – 19.03 g OC m^{-2} yr^{-1}) and limited to ponds displaying an initial establishment of *L.riparium* and *Agrostis*.

Based on results from the chapter V, burial rates have been adjusted to factor in this time constrained element governing OC burial. Results can be seen in figure VI.13, which displays adjusted burial rates for the ponds. Adjusted OC burial rates ranged from 78.5 ± 10.28 to 247.2 ± 32.36 g OC m^{-2} yr^{-1} and displayed an overall site average of 142.44 ± 18.65 g OC m^{-2} yr^{-1}, similar to values reported in Gilbert et al. 2014.

Figure VI.13: Adjusted burial accounting for low levels across the first 3 years from creation.



Establishment of *L.riparium* promoted damp and anoxic conditions within the bottom soil promoting the formation of sediment and establishment of rooted macrophytes (Jeffries, 2008; Observations Chapter V). It is possible that the establishment of *L.riparium* promotes the preservation and burial of OC. Coupled with the establishment and growth of vascular rooted macrophytes, made of more recalcitrant OM, it is likely, that it is the mid stages of vegetation succession where OC burial becomes significant.

4.7 Comparison to Other Ecosystems

Table VI.4 highlights OC burial rates observed in the ponds from this study in comparison to a range of other global ecosystems. The small experimental ponds exhibit burial rates elevated above those observed in terrestrial and larger natural aquatic environments, exceeded only by rates observed in reservoirs, impoundments and marine vegetated areas (Downing, 2010).

OC burial rates are elevated substantially above those observed in any other terrestrial ecosystem. The coverage and extent of terrestrial ecosystems such as forests and peatlands means that despite considerably lower burial rates of OC they are still deemed as globally significant sinks of atmospheric carbon and rightly so (Houghton, 2010; IPCCC, 2007). However, given the magnitude of burial in these small ponds and revised estimates of their global coverage it may be that these systems contribute more to global carbon cycling than previously thought and could be utilised in land sparing strategies to offset GHG emissions.

Table VI.4: Carbon burial in a range of environments. Data for lakes and impoundments are from *Mulholland and Elwood (1982),* data for terrestrial ecosystems including peatlands are from *Schlesinger* (1997), data for marine ecosystems are from *Duarte et al.* (2005), data from Aquaculture ponds taken from Boyd (2010), Data for Wetlands taken from Mitsch (2013), Data for small impoundments Pittman (2013), data from temperate reservoirs Sobek (2010). Data in red taken from this study. (Adapted from Downing et al. 2008).

Environment[a]	Mean OC Burial[b] rate ($g\ m^{-2}\ yr^{-1}$)	Range
Agricultural Impoundments	2122	148 - 17,392
Temperate Reservoir	1113±482	536 - 1950
Impoundments (Asia)	980	20 - 3300
Impoundments (Central Europe)	465	14 - 1700
Impoundments (United States)	350	52 - 2000
Impoundments (Africa)	260	
Small Impoundments (Missouri)	236.75	183 - 279
Aquaculture Ponds	148.9±90.3	28 - 333
Small Experimental Ponds (3 year adj)	142.44±15.04	78.5±8.29 – 247.2±26.16
Marine Vegetated Habitats	139	83 – 151
Small Experimental Ponds (this study)	122.10±13.89	67±7.11 - 212±22.42
Wetlands	118	42 - 306
Small Mesotrophic Lakes	94	11 – 198
Abandoned Agricultural land, Returning to grassland	56	1.6 - 110
Mine Spoils Returning to Forest and Grassland	42	28 - 55
Peat lands	31	8 – 105
Marine Depositional Areas	31	17 – 45
Abandoned Agricultural Land Returning to Forest	30	21 - 55
Small Oligotrophic Lakes	27	3 – 128
Large Mesotrophic Lakes	18	10 – 30
Large Oligotrophic Lakes	6	2 – 9
Boreal Forest	4.9	0.8 – 11.7
Temperate Forests	4.2	0.7 – 12
Tropical forests	2.4	2.3 – 2.4
Temperate grassland	2.2	
Tundra	1.2	0.2 – 2.4
Temperate Desert	0.8	

OC burial rates in water bodies associated with human activity such as agricultural impoundments and aquaculture ponds, were elevated above OC burial rates observed in this study (Boyd, 2010; Downing, 2010; Downing et al., 2008). This may arise from large inputs and delivery of allocthonous OM entering impoundments and reservoirs that are often stream fed. Coupled with high rates of autochthonous productivity and enhanced nutrient concentrations, particularly in agricultural catchments, this can lead to greater preservation of OC, resulting in considerably higher OC burial rates.

Burial rates of OC also appear greater in aquaculture ponds. Anthropogenic activities on these systems, likely contribute to enhanced rates of OC burial. Many aquaculture ponds are subject to the addition of feedstock and fertilisers (Boyd, 2010) with nutrient concentrations often artificially enhanced to improve productivity. Another important consideration of OC burial in aquaculture ponds is the fate of accumulated sediment. In many cases ponds are drained routinely at the end of the growing season, oxidising sediments, others are dredged to maintain integrity of the aquaculture process (Boyd, 2010). Although OC burial is high, its storage may be negligible in such systems, given the turnover of stored OC due to management practices.

Although constructed, the ponds in this study have been left to develop naturally in a nature reserve, away from agricultural spraying and run-off from fertilisers. These ponds therefore represent semi-natural systems yet still have OC burial rates close to, if not exceeding rates observed in aquaculture ponds. If the range of burial rates observed across environments is considered, we can see that these ponds have the potential to bury more carbon than Impoundments and reservoirs, particularly if we factor in negligible OC burial across the first 3 yrs of pond succession.

When we compare OC burial with natural freshwater habitats such as mesotrophic and oligotrophic lakes, it is apparent that small ponds have the capacity to bury OC at much greater rates. In comparison to wetlands, well known and documented sites of intense carbon cycling and burial, the small $1m^2$ ponds in this study exhibited comparable, if not elevated OC burial rates (Mitsch et al., 2013; Kayranli, 2011). This suggests that small constructed ponds have the ability to be some of the most productive and important freshwater habitats for OC burial across the globe, providing further evidence to support Downing's (2010) hypothesis that OC burial in smaller water bodies is disproportionately intense.

Sampling was limited to a single site and therefore does not represent the heterogeneity of ponds at landscape, national and global scales. However, results are extremely insightful; providing a unique understanding of rates of OC burial in small constructed ponds in temperate lowland areas. Levels of OC storage are comparable to those observed in a range of pond types at both a regional and national level (Gilbert 2011 thesis), and OC burial rates are some of the highest recorded for natural ecosystems (Gilbert et al., 2014), representing a size category of water bodies previously overlooked by limnologists (Downing, 2010). Further studies should be undertaken to quantify OC burial in small ponds, given their potential significance in global carbon cycling and prospective use as natural options in landscape carbon mitigation strategies.

4.8 Implications for the Global Carbon Cycle

Latest estimates suggest inland water bodies <0.001 km^2 contribute considerably to atmospheric CO_2 and CH_4 emissions (Holgerson & Raymond, 2016; Raymond, 2013). Holgerson & Raymond (2016) suggests that of the estimated 0.583 Pg C yr^{-1} released from global non-running waters, very small ponds account for 15.1% of CO_2 emissions and 40.6% of CH_4 emissions, despite occupying only 8.6% of the total areal coverage. It is estimated that ponds release 0.091 Pg C yr^{-1} globally, or 167.02 g C m^{-2} yr^{-1}, as both CO_2 and CH_4 emissions (see table VI.5 for tabular breakdown of results).

However, most studies fail to compare estimated emissions to OC burial rates, which are needed to determine whether these systems function as a net sink or source. This is likely due to limited available estimates and methods available to produce accurate figures. Available studies have outlined disproportionate rates of biogeochemical cycling in relation to larger water bodies, but are limited to specific pond types or climatic zones. For instance, Holgerson & Raymond (2016) used emission data for ponds <0.001 km^2 that were located in sub-arctic zones, comprising largely of permafrost thaw ponds, which undoubtedly function differently to ponds in temperate and tropical zones. Boyd (2010) produced burial rates for ponds but the study was limited to aquaculture ponds and Downing (2008) was restricted to agricultural impoundments.

Table VI.5: Tabular breakdown of flux data in comparison to burial data. Flux data taken from Holgerson & Raymonds 2016. Burial rates used from this study have been extrapolated using areal coverage used in Holgerson & Raymond, 2016.

Emissions (Holgerson & Raymond, 2016)	CO_2	CH_4	Total
Mmol C m^{-2} d^{-1}	35.18	2.28	
g C m^{-2} yr^{-1}	154.40	12.63	**167.02**
Total release from lakes and ponds Pg C yr^{-1}	0.571	0.012	
% of C release from waterbodies <0.001 km_2	15.1	40.6	
Pg C yr^{-1}	0.086	0.0049	0.091
Burial (This Study)			
OC Burial g C m^{-2} yr^{-1}			**142.44**
Total burial Waterbodies <0.001 Pg C yr^{-1}			0.072
Flux			
Net Flux g m^{-2} yr^{-1}			**24.58**
Net Flux Pg C yr^{-1}			**0.019**

The lack of published studies addressing the overall net balance of carbon fluxes, largely arises from the lack of comprehensive global data quantifying rates of both OC burial and emission. Factoring in OC burial rates is a crucial component when stating with confidence whether these systems act as a net source or sink of carbon. Although subject to limitations and highly unrepresentative of ponds globally, estimated rates of OC burial in this study have been assessed against the most recent published emission values in Holgerson (2016).

Data from these studies suggest ponds emit 167.02 g C m^{-2} yr^{-1}. In comparison to burial rates of 142.44 g C m^{-2} yr^{-1}, this would indicate a net source of 24.58 g C m^{-2} yr^{-1}. Up scaled globally figures would be around 0.091 Pg C yr^{-1} released, compared to burial of 0.072 Pg C yr^{-1}, indicating a net emission of 0.019 Pg C yr^{-1}. This would suggest that these systems may not be a substantial source, as previous studies have suggested, and that when factoring in burial rates, differences between emission and burial rates is not so considerable.

The margin of difference observed between emission and burial rates of OC is small (0.019 Pg C yr^{-1}). Given the range of burial rates observed, it is possible for this value to change to a net sink of 0.037 Pg C yr^{-1} using upper threshold estimates. These values should not be treated as solid evidence to support the theory that ponds are a definitive net source or sink of carbon to the atmosphere. They should however, be used to act as a springboard for further research aiming to quantify and constrain the net balance between OC burial and emissions from ponds, particularly due to recent research highlighting the complexity of carbon fluxes and biogeochemical cycling. Of high interest is their behaviour as both a source and sink, under different environmental conditions, such as transitional hydro-periods (Gilbert et al., 2016).

Furthermore, the residence time of some small ponds over long geological time scales, such as Kettle Holes and Pingo Ponds in Norfolk, raises the question of whether these systems may reach a state of climate controlled equilibrium, whereby OC burial and emission are balanced out over a longer time scales (Reverey et al., 2016). Whether or not this is the case, results from this study suggest that ponds have the capacity to store large amounts of OC over relatively short time scales, raising the question of whether ponds could be constructed to act as an ecological buffer against rising CO_2 levels or mitigate emissions from the agricultural industry under land sparing strategies.

4.9 Carbon Mitigation Potential

Given the magnitude of OC burial in these small aquatic systems in comparison larger water bodies and terrestrial ecosystems, they have undoubted potential for inclusion as natural tools in landscape carbon mitigation strategies. Assessing their potential at a national scale, burial rates have been extrapolated using data on UK coverage of ponds (Williams, 2007). Although the study omits ponds <25 m^2, here we estimate that ponds could potential capture 0.04 Mt C yr^{-1}, which could be considerably more including ponds <25m^2. Converted to CO_2e this would equate to ~0.15 MtCO_2e yr^{-1}. See table VI.6 for a tabular breakdown of data. Overall UK GHG emissions for 2014-2015 were estimated to be around 523.1 Mt CO_2e yr^{-1} (DECC report 2015 UK Greenhouse Gas Emissions), of which ponds have the potential to mitigate up to 0.028% of total emissions. Emissions of GHG from UK agriculture in 2013 were 56.1 Mt CO_2e (DECC, 2015) to which current pond stocks could mitigate 0.27%.

Table VI.6: Estimated national annual burial rates of Carbon from small ponds based on size distribution and coverage data from the Countryside survey 2007.

Pond Size Category (m^2)	25-400	400-2000	2000-10000	10000-20000
Number of Ponds	332,500	117,800	26,500	4,100
Median size of ponds in size range (m^2)	140	800	3,000	14,500
Total area of ponds in size category (m^2) (number x median)	46,550,000	94,240,000	79,500,000	59,655,000
Potential Carbon burial (T OC yr^{-1})	6628.7	13419.8	11320.8	8494.9
Total OC Burial T C yr^{-1}	39864.12		**0.04 Mt**	

Following on from COP21 and targets outlined under the conference legislative guidelines; the agricultural industry has agreed to reduce emissions by 3MtCO_2e per year by 2020 as part of the industry action plan (Lamb et al., 2016). This equates to 0.817 Mt OC, which would be potentially covered by the creation of an extra 0.57 M ha (3.3% of total agricultural area) of small ponds throughout UK. Interestingly this is similar to the 0.7Mha proposed for the restoration of peatland and wetland, alongside increasing forest cover from 12% to 30%, by Lamb (2016) in assessment of the potential of land sparing to offset greenhouse gas emissions from agriculture.

The UK land surface occupies around 24.4 million hectares (Mha). Agriculture occupies around 17.2 Mha, whilst freshwater currently only occupies around 0.25 Mha. Areas of freshwater could be increased by 0.57 Mha to 0.82 Mha through the construction of

ponds in areas of grassland, pasture, rough grazing and around cropped areas. This represents a conversion of around 3.3% of total agricultural area to small ponds. This may seem excessive given the current and future demands on agricultural land for food production. However, the small size and versatility of these features coupled with their ease of construction would allow ponds to be created in areas of all manner of land use with little effect on crop yields or agricultural economy. If ponds were constructed in strategic networks to attenuate floodwaters, it is possible that a loss in crop yield from the conversion of farmland to ponds could be offset by securing crops from flood damage.

It is possible that future landscape management practices could include and encourage the construction of networks of these features. Ponds could be engineered in both an ecologically and economically efficient manner in order to provide a whole raft of ecosystem services. In a regulatory capacity, they can mitigate some of the largest problems currently faced by UK agriculture in particular carbon mitigation, flooding, soil erosion and watercourse pollution.

5.0 Conclusion

Results from this chapter confirm that OC burial within small constructed ponds is substantial. In comparison to other ecosystems, rates were elevated beyond those documented for terrestrial environments and larger water bodies. OC burial was most comparable to aquaculture ponds, despite not receiving the same management and artificial enhancement of productivity that these systems have. Results also demonstrate that vegetation community succession, particularly the earlier establishment of *L.riparium* and coverage of *J.articulatus*, plays a significant role in determining overall OC storage and burial. This would also support the observation in chapter V, which demonstrated that earlier establishment of *L.riparium*, resulted in the development of more characteristic sediment profiles. This is a key result. It demonstrates the potential to naturally engineer ponds through vegetation to enhance their OC burial capacity, bypassing initial stages off succession where OC burial is low.

From a global perspective, in comparison to recently published emission rates, which suggest very small ponds release substantial amounts of CO_2 to the atmosphere, the OC burial rates identified here demonstrate that the majority of this release would be offset by OC burial. The heterogeneity of small freshwater systems and variable hydro-period in relation to climate complicates attempts to comprehensively quantify the sink/source balance at global scales, but the results here demonstrate that the balance may be much finer than has recently been suggested.

Key Findings:

1. Calculate OC storage and burial rates for the Hauxley ponds.

- Estimated OC storage values for sampled ponds ranged from 1413 ± 184.96 to 4459 ± 583.68 g OC in a whole pond with an overall site average of 2564 ± 335.63 g OC. It is estimated that cumulatively the 30 small experimental ponds store 76.92 ± 10.06 Kg OC.
- OC burial rates ranged from 67 ± 8.77 to 212 ± 27.75 g C m^{-2} yr^{-1} with an average of 122.10 ± 15.98 g C m^{-2} yr^{-1}.
- Adjusted burial rates, based on the negligible OC burial observed the first three years highlighted in chapter V, ranged from 78.5 ± 10.28 to 247.2 ± 32.36 g OC m^{-2} yr^{-1} and displayed an overall site average of 142.44 ± 18.65 g OC m^{-2} yr^{-1}.

2. Identify significant differences in OC storage and burial between ponds

- Group 3 ponds stored significantly more OC than the group 1 ponds.

3. Identify the impact of vegetation community succession on OC storage and burial

- The earlier colonisation and establishment of *L.riparium* and *G.fluitans* resulted in significantly higher OC storage and burial rates.
- Ponds dominated by *J.articulatus* and retaining *C.vulgaris* for longer periods had stored significantly less OC.

4. Explore the implications of OC burial in a national and global context

- Upscaled to a National level these values would suggest that ponds have the potential to be implemented as ecological tools in carbon mitigation strategies, through land sparing and CAP greening schemes.
- Globally up-scaled estimates suggest that the sink/source balance of carbon fluxes in small water bodies may be much finer than has been recently suggested.

Chapter VII – Microbial Ecology: Who's there? Implications for Carbon Burial

Research Objectives:

1) Identify dominant microbial communities present within the ponds
2) Assess variations in microbial diversity and community structure between different ponds
3) Identify relationships between microbial community structure, sediment physicochemistry and vegetation coverage
4) Explore the implications for OC storage and carbon cycling

1.0 Introduction

The role of microbes as primary degraders of OC to either CO_2 or CH_4, in small water bodies, is of increasing interest given the latest estimates of GHG emissions from these systems. Understanding microbial community composition in very small ponds, which contribute a large proportion of overall emissions from inland waters (Holgerson & Raymond, 2016), is particularly important. However, large uncertainties exist on the communal role of microbes in aquatic GHG cycling, despite some studies documenting the significant effect of microbial community composition on ecosystem process rates.

High throughput sequencing using 16s rRNA, facilitates the rapid analysis of microbial communities at a much higher throughput than has previously been possible (Wang et al., 2012). The easier characterisation of microbial community structure across different ecosystems and environmental gradients has also led to the identification of novel phylotypes, previously thought absent in certain environments. This has led to the identification of several *Actinobacteria* and *Betaproteobacteria* phylotypes that appear to be characteristic and abundant in freshwaters (Briee et al., 2007).

However, knowledge of the composition of microbial communities in freshwater systems is fragmented at best, considering the substantial heterogeneity of these habitats. Complex seasonal and climate driven hydrological dynamics (Reverey et al., 2016; Hahn, 2006), alongside various ecological factors, both physicochemical (size, water chemistry, retention time, temperature, irradiation) and biological (organic matter supply, primary producers, predation, viral dynamics), influence the composition of microbial communities (Zhang et al., 2015; Song et al., 2012; Briee et al., 2007).

An increasing number of studies have focused on wetland environments (Ansola et al., 2014; Peralta et al., 2013, Wang et al., 2012) and freshwater lakes (Chen et al.,2015; Zhang et al., 2015; Song et al., 2012). A smaller number have focused on smaller ponds (Briee et al., 2007). These studies have identified that *Proteobacteria* are the most dominant phylum in freshwater sedimentary environments alongside *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Chlorobi*, *Firmicutes*, *Gemmatimonadetes*, *Planctomycetes* and *Verrucomicrobia*. However, little is known about the ecological function of groups of freshwater bacteria, largely due to lack of genomic insights into the metabolic capacity (Hahn, 2006). Table VII.1 provides an overview of phylum known to dominate freshwater sediments and some of the known ecosystem functions associated with them.

Table VII.1: Overview of dominant freshwater bacterial phylum

Phylum	Overview	Reference
Proteobacteria	Contain a large degree of bacterial metabolic diversity, linked to numerous biogeochemical functions, related to global carbon, nitrogen and sulphur cycling Widely distributed in freshwater sedimentary environments	Zhang et al., 2015; Ansola et al., 2014
Subclasses		
Alpha	Widely distributed in freshwater sediment samples and have shown a strong association with pH and nutrient concentrations	Peralta et al., 2013
Beta	Widely distributed in freshwater sediment samples and have shown a strong association with pH and nutrient concentrations	Peralta et al., 2013
Delta	Widely distributed and are largely involved in sulphur reduction in anaerobic conditions	Wang et al., 2012
Gamma	Widely distributed and are largely involved in sulphur reduction Facultative anaerobes and some obligate aerobic species. Recent study suggests they account for 40 to 70% of CO_2 fixation using sulphur as an electron donor in intertidal sediment	Dyksma et al., 2016 Wang et al., 2012
Epsilon	Occur at high abundance at the oxic anoxic interface within sediment environments where it plays a key role in nitrogen and sulphur cycling	Grote et al., 2011
Acidobacteria	Abundant members of bacterial communities within soils Few strains have been fully described Role within freshwater sediment has not been well documented	Newton et al., 2011 Peralta et al., 2013
Bacteroidetes	*Bacteroidetes* have been observed in high abundance within wetland sediment samples Known for degrading high molecular weight compounds in natural environments	Ansola et al., 2014 Newton et al., 2011
Actinobacteria	Play an important ecological role in natural ecosystems Ability to degrade a variety of environmental chemicals and contaminants Ability to withstand drought periods through spore formation	Zhang et al., 2015 Fuentes et al., 2014 Cupples 2013
Chloroflexi	Linked to dechlorination of chlorinated organic chemicals Ubiquitous in natural environments, and have been observed a variety of freshwater sediment samples Role of Chloroflexi within freshwater sediments is still uncertain Found to dominate anaerobic sediment layers in estuary environments	Zhang et al., 2015 Lucheta et al.,2013 Ansola et al., 2014 Liu et al., 2009
Verrucomicrobia	Ubiquitous in soils Abundant and active group of soil bacteria role within sedimentary environments is not well understood	Zhang et al., 2015 Sangwan et al., 2005

Microbial functions broadly involved in GHG cycling include carbon degraders, phototrophs, methanotrophs and methanogens. Strickland et al. (2009) reported a ~20% variation in the total amount of carbon remineralised from litter decomposition, attributed to changes in microbial community composition. However, there is currently debate around assumptions that the environment ultimately controls process rates. Some speculate that microbial communities exert a proximate control on process rates but are ultimately structured by the contemporary environment, whereas contradictory views suggest microbial community composition (i.e. the whole community genotype) may, in combination with the environment, ultimately (not just proximally) determines ecosystem process rates (Strickland et al., 2009; Reed and Martiny, 2007; Balser & Firestone, 2005).

The number of studies investigating microbial diversity in inland waters is far too low relative to the number of different types of inland waters (Hahn, 2006), to even provide an overview of the microbial diversity within these habitats. Studies focusing on ponds have largely focused on anoxic systems (Briee et al., 2007) or permafrost thaw ponds (Neghandi et al., 2014).

Observed rates of productivity, carbon emissions and burial within small temporary ponds have been identified as being disproportionately intense and complex in relation to hydroperiod (Gilbert et al., 2016; Previous chapters. Emerging research is indicating that small ponds switch from sizable sinks to substantial sources of carbon over a matter of days, with carbon remineralisation becoming notably intense as ponds transition from wet to dry (Reverey et al., 2016; Gilbert et al., 2016; Catalán et al., 2014). Reverey et al. (2016) also reported that sustained dry periods are lethal to microorganisms and can result in the killing of >70% of the microbial biomass. Microbial activity within these systems is intense and undoubtedly complex, responding dynamically to changing hydrological and environmental conditions. However, limited research has characterised microbial communities within small constructed temporary ponds in temperate lowland landscapes.

This chapter aims to; 1) provide a snapshot survey on the microbial community structure and diversity in sediments from a number of constructed ponds 2) Assess variability between different ponds, 3) Identify relationships between microbial community structure, sediment physicochemistry and vegetation coverage and 4) Explore the implications for OC burial and carbon cycling. Both mature (>20yrs) and new (<4yrs) ponds have been sampled to identify differences in community structure in ponds at different stages of succession and assess how this may potentially relate to OC storage and cycling dynamics within these highly active systems.

2.0 Methods

See Chapter 3 for detailed discussion on methods.

3.0 Results

3.1 Alpha Diversity

The number of phylotypes in each sediment core sample was considered as the number of operational taxanomic units (OTU's) (Wang et al., 2012). The number of OTU's obtained depends on the phylogenetic distance considered. In this study a distance of 0.03 was used (Kozich et al., 2013).

Diversity was calculated using a sub-sample of 20,000 sequence reads across all observed OTU's. Number of sequence reads was plotted against the number of observed OTU's to produce rarefaction curves, which can be observed in Figure VII.1 and VII.2**.** The Good's coverage estimator (Kozich et al., 2013) reveals that on average 96% (94% - 99%) of OTU's were obtained in all the sediment samples at a distance of 0.03 indicating the majority of OTU diversity has been captured.

Figure VII.1 displays rarefaction curves for each sediment core. Down core trends in diversity were not uniform across ponds but were more similar within groups of ponds (Group 1 – ponds last to develop extensive coverage of *L.riparium* and terrestrial species; Group 2 – Intermediate group; Group 3 – ponds first to develop extensive swards of *L.riparium* and establishment of *G.fluitans*). In group 3, diversity was generally higher in the upper layers and decreases down the core profile. In group 1, diversity was generally higher within the lower sediment core layers, whereas in group 2, down core trends were less consistent across the ponds.

Overall diversity was generally higher within the group 3 ponds, particularly ponds 28 and 27. Group 2 ponds also displayed higher diversity in general than the group 1 ponds, apart from pond 22. Figure VII.2 displays rarefaction curves for the average diversity observed across the top 5cm of individual pond cores. Results suggest that patterns of increasing diversity somewhat reflect patterns of OC storage observed between ponds in chapter VI.

Figure VII.3 displays rarefaction curves for the newly constructed ponds. ST3 displayed the highest diversity followed by ST2 then ST1. This follows the same spatial patterns across the site as the mature ponds in figure VII.2. This would also follow the same patterns of OC storage, observed in the new ponds.

Figure VII.1: Rarefaction curves for 20000 subsamples. Curves plotted are for the full sediment core of individual ponds.

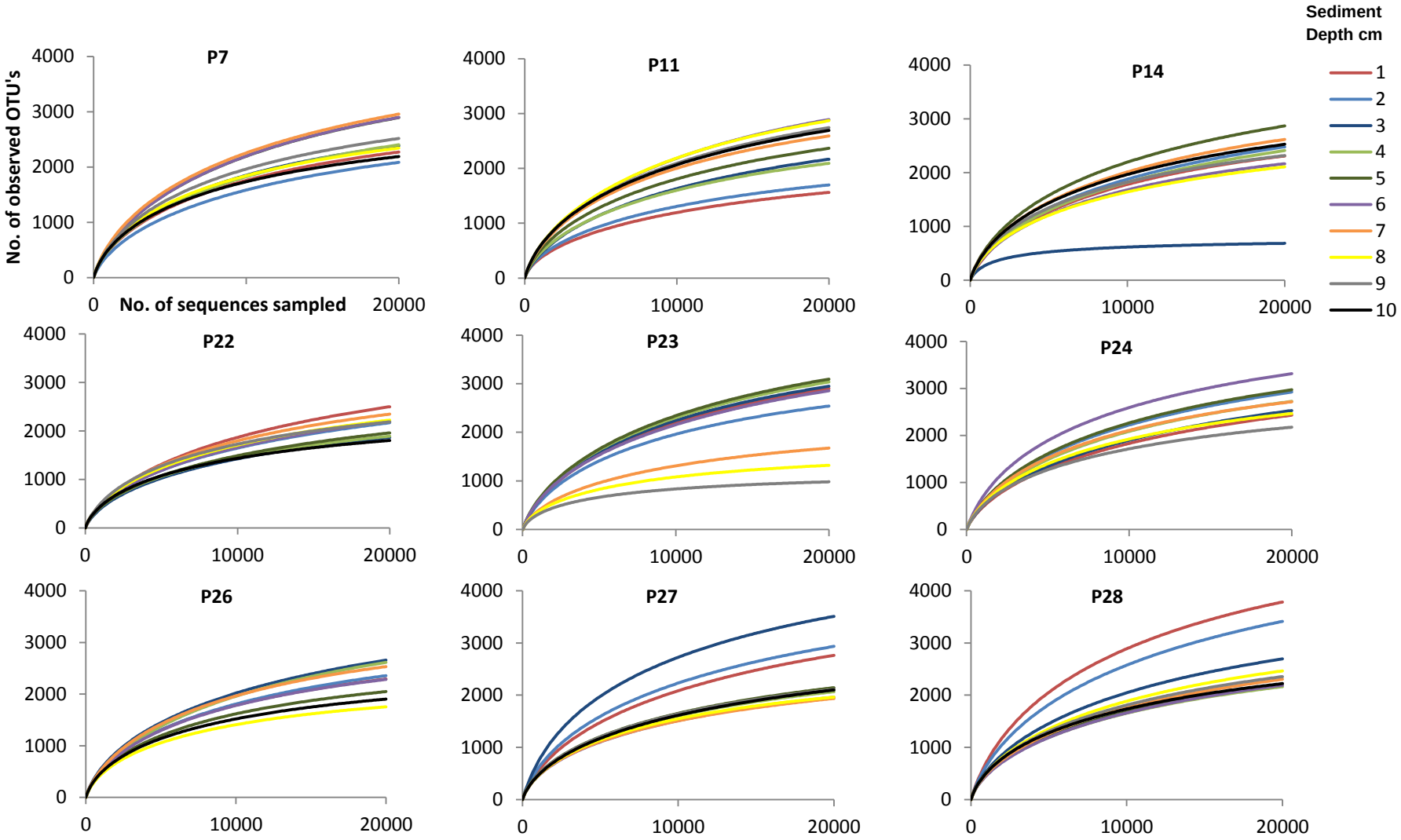


Figure VII.2: Rarefaction curves for 20000 OTU subsamples. Curves plotted are average number of sequence reads observed across the top 5cm.

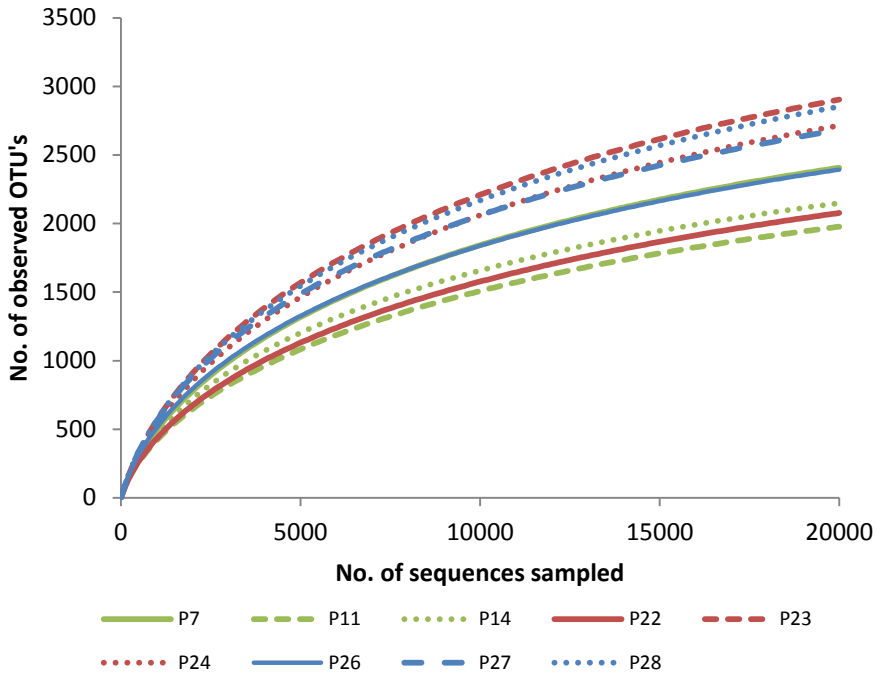
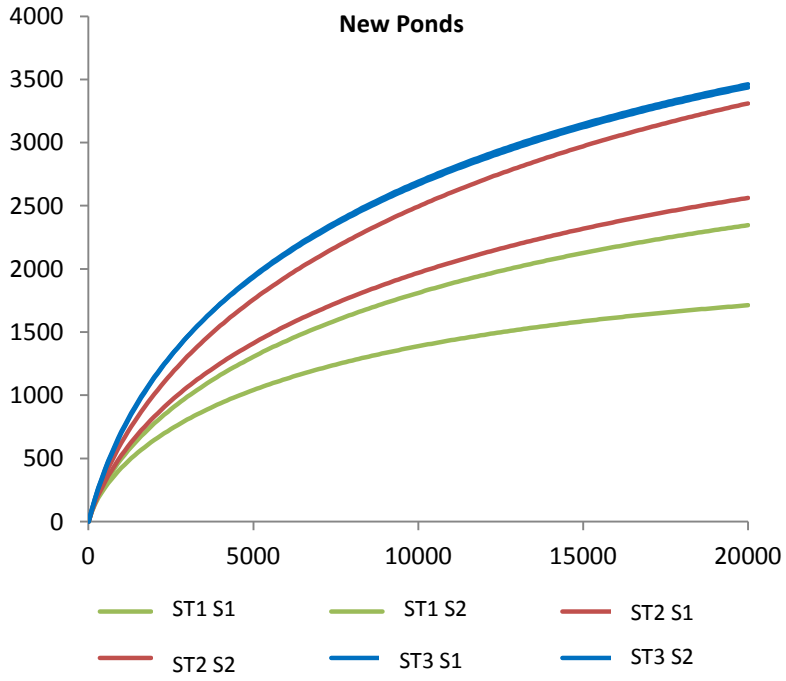


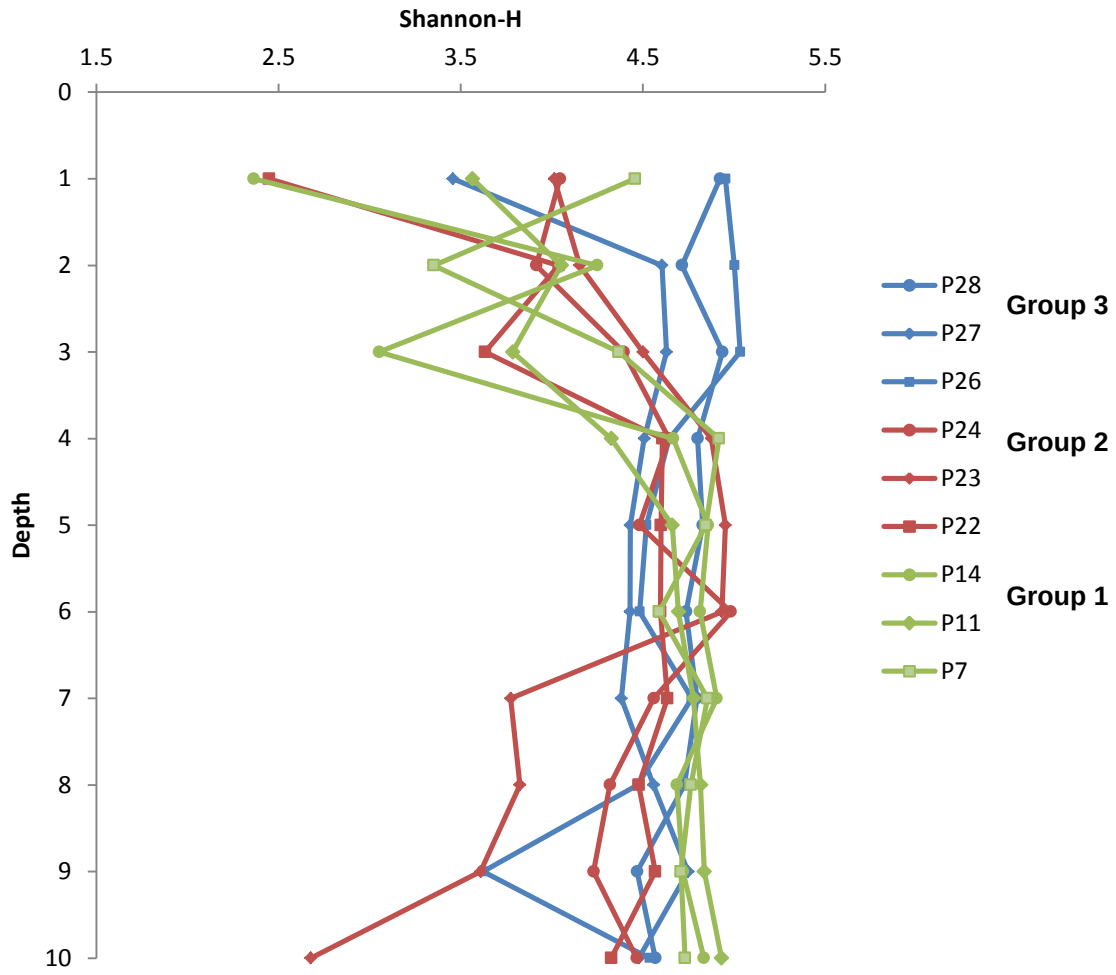
Figure VII.3: Rarefaction curves for 20000 OTU subsamples. Curves plotted are average number of sequence reads observed across the new ponds.



Alpha diversity indices of the 96 samples (Individual 1cm core slices) were also calculated, see figure VII.4 which displays Shannon diversity values for each core profile. Simpson's diversity index and the Chao 1 richness estimator were also calculated and showed similar trends to Shannon diversity values.

Patterns down the core profile were observed, with diversity generally increasing with core depth. Variations in Shannon Diversity values between pond groups were also observed, with groups 3 displaying higher diversity in upper layers in relation to groups 1 and 2. Group 1 ponds demonstrated higher diversity in the lower layers, indicating contrasting down core diversity patterns between different pond groups. Patterns follow the same spatial variation observed in OC between ponds, higher in ponds 27 and 28, decreasing substantially in group 1 ponds and pond 22.

Figure VII.4: Shannon Diversity values plotted for individual sediment cores.

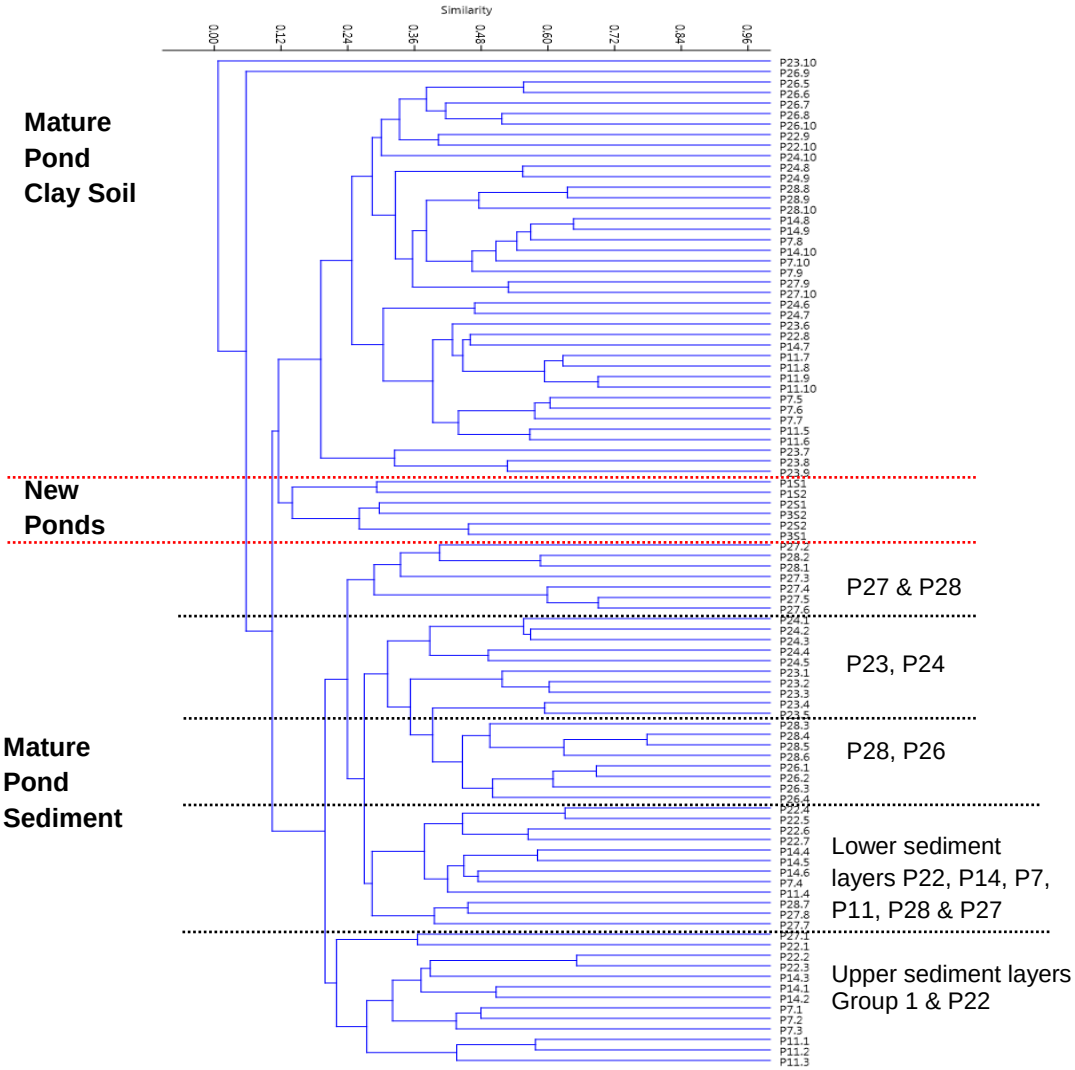


3.2 Beta Diversity

3.2.1 Cluster analysis

Cluster analysis was performed using PAST v3.1 (Hammer et al., 2001)**.** Figure VII.5 displays a cluster dendogram for samples based on the paired grouping method and using Bray-Curtis as a measure of similarity. Clustering was largely separated into two broad categories; upper and lower sediment layers. These layers somewhat reflect depth determined in chapter VI. The newly constructed ponds fell in middle of the two but belonged to the cluster of lower sediment layers. Clustering of pond groups was also apparent within sediment layers. Group 3 and group 2 ponds (P23 & P24) were more similar in terms of bacterial community structure, than upper sediment layers in group1 and P22. Upper layers in group 1 and P22 were more similar to the lower sediment layers of all groups.

Figure VII.5: Cluster dendogram for mature and new ponds. Clustering performed in Past v3.1 using single linkage method with Bray-Curtis applied as a distance measure.



3.2.2 Bacterial Community Structure

Figure VII.6 highlights the bacterial phylum observed across all samples and the associated number of sequence reads. Major phyla observed were *Acidobacteria*, *Actinobacteria, Bacteroidetes, Chloroflexi*, *Verrucomicrobia* and *Proteobacteria*. *Proteobacteria* were the most dominant phylum observed in all samples, of which the *Alpha*, *Beta*, *Delta* and *Gamma* subdivisions were most abundant. Less dominant phyla observed were, *Chlorobi*, *Planctomycetes*, *Spirochaetes*, *Nitrospira*, and *Firmicutes*. The relative abundance of these phyla can be observed in figure VII.7.

Figure VII.6: Number of sequence reads observed for specific bacterial phylum. Proteobacteria has been included as both the whole class and sub-classes.

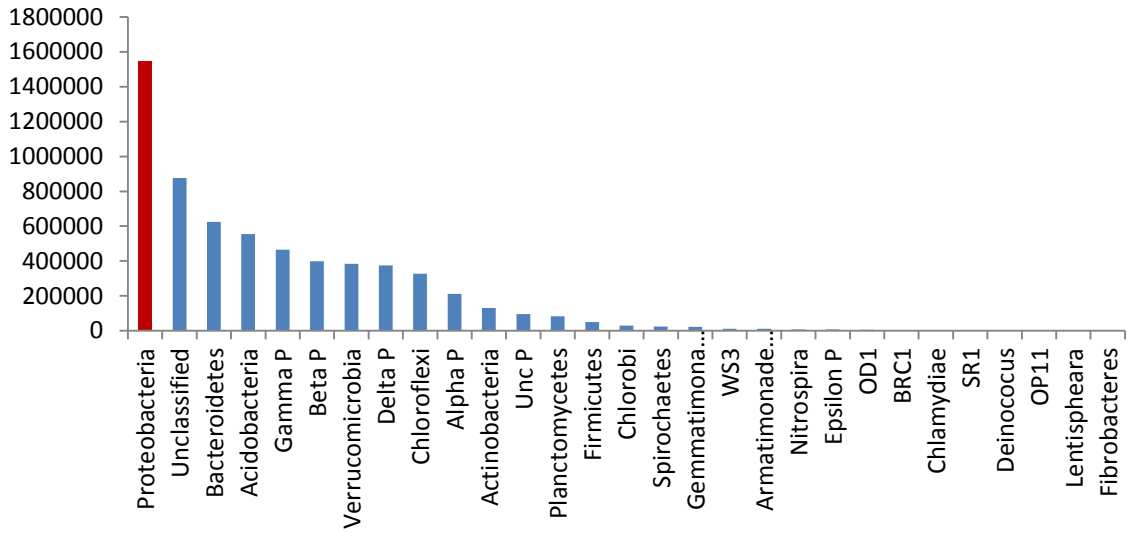
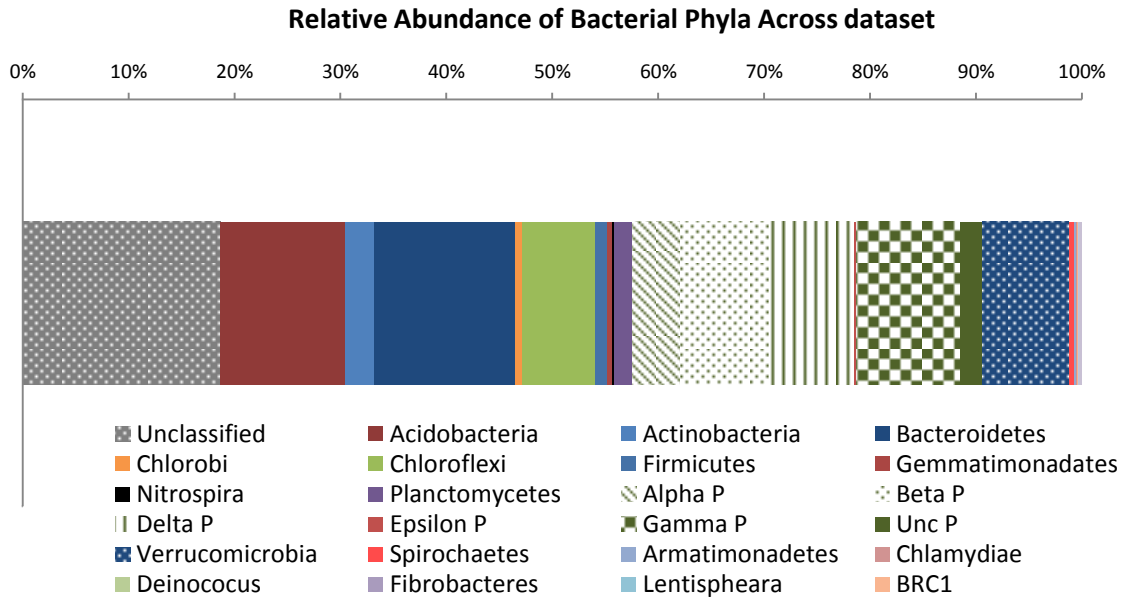


Figure VII.7: Relative abundance of bacterial phylum observed across all samples.



3.2.2i Mature ponds

Relative Abundance of bacterial phylum observed across ponds and within individual core profiles, can be observed in figure VII.8. General down core patterns were observed across most ponds. Phyla displaying decreasing abundance down the core profile were *Actinobacteria*, and *Bacteroidetes*. *Chloroflexi* abundance increased considerably with depth, whilst abundance of *Acidobacteria* and *Verrucomicrobia* was fairly consistent down the core profile. *Proteobacteria* abundance was less consistent with depth; Group 1 ponds displayed decreasing abundance with depth, Group 2 ponds displayed decreasing abundance, prior to slight increase in some lower core layers, and Group 3 ponds displayed relatively consistent abundance, albeit relatively low.

Variability in *Proteobacteria* sub-class abundance across samples was also observed, particularly noticeable for *Beta*, *Delta* and *Gammproteobacteria.* Relative abundance and down core patterns were not consistent between pond groups*. Gammaproteobacteria* were generally more abundant in upper sediment layers across group 2 and group 1. Abundance generally decreased down the core profile, although subsequent increases in abundance within lower layers in group 2 ponds are apparent. *Gammaproteobacteria* were less abundant within group 3 ponds and were relatively consistent with depth overall. *Beta* and *Deltaproteobacteria* were generally more abundant in group 1 ponds. In the majority of ponds, they displayed a general increase in abundance down the core profile.

Other dominant phylum displaying variability between ponds include*; Chloroflexi,* which appeared more abundant in group 1 relative to other ponds, and *Actinobacteria*, *Bacteroidetes*, *Acidobacteria* and *Verrucomicrobia*, which were more abundant in group 3 ponds.

3.2.2ii Mature and new ponds

Relative abundance of bacterial phylum across new ponds in comparison to the average relative abundance of the top (2 cm) sediment layers and bulk (full core mean) of mature pond sediment can be seen in figure VII.9. Marked differences can be observed between the ponds*. Actinobacteria*, *Acidobacteria* and *Chloroflexi* were more abundant in the new ponds. However, the most considerable difference observed between mature and new ponds was abundance of *Proteobacteria*, which were markedly less abundant in the new ponds.

Figure VII.8: Relative Abundance of Bacterial Phylum down the core profile of individual ponds.

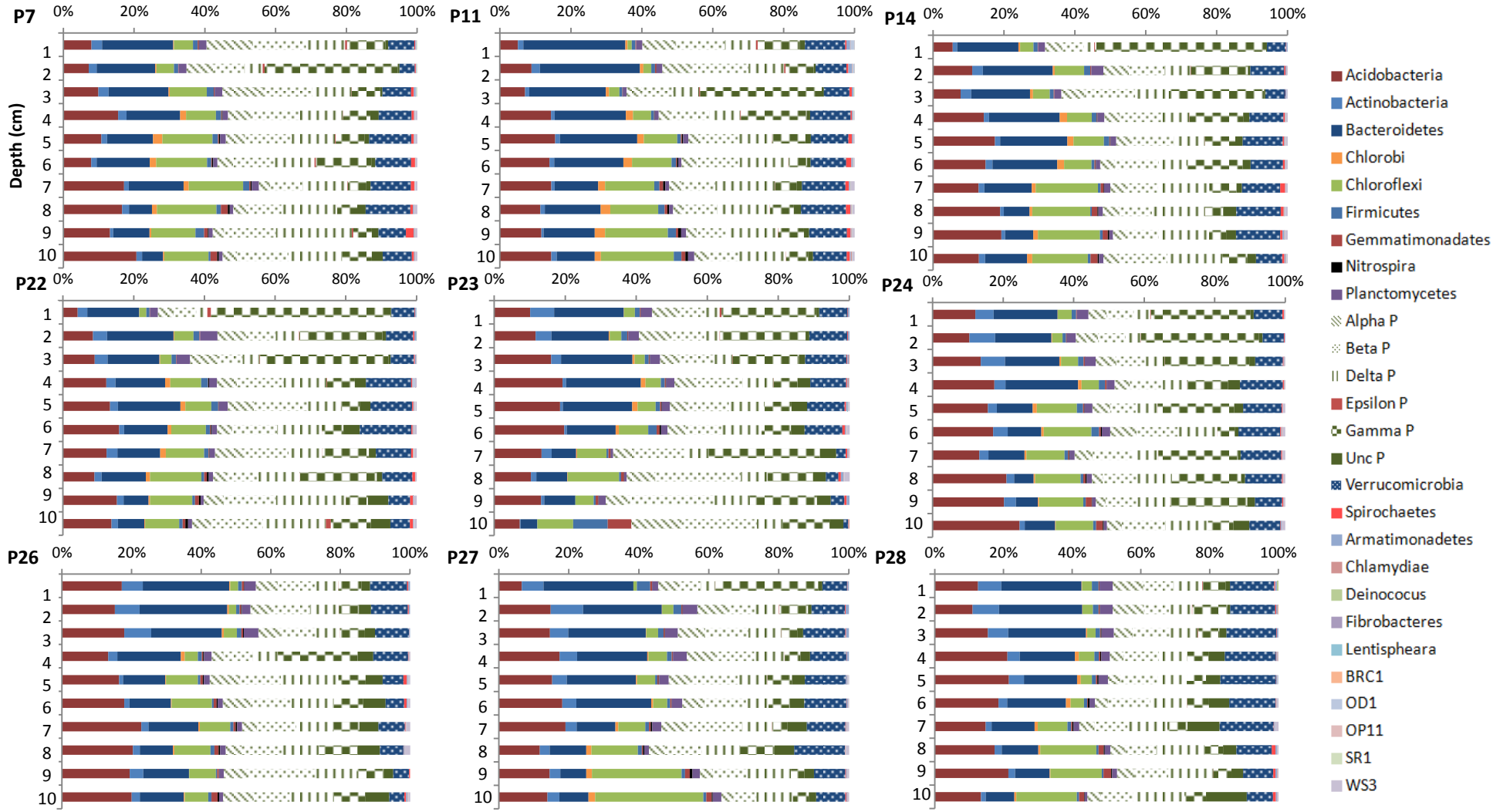
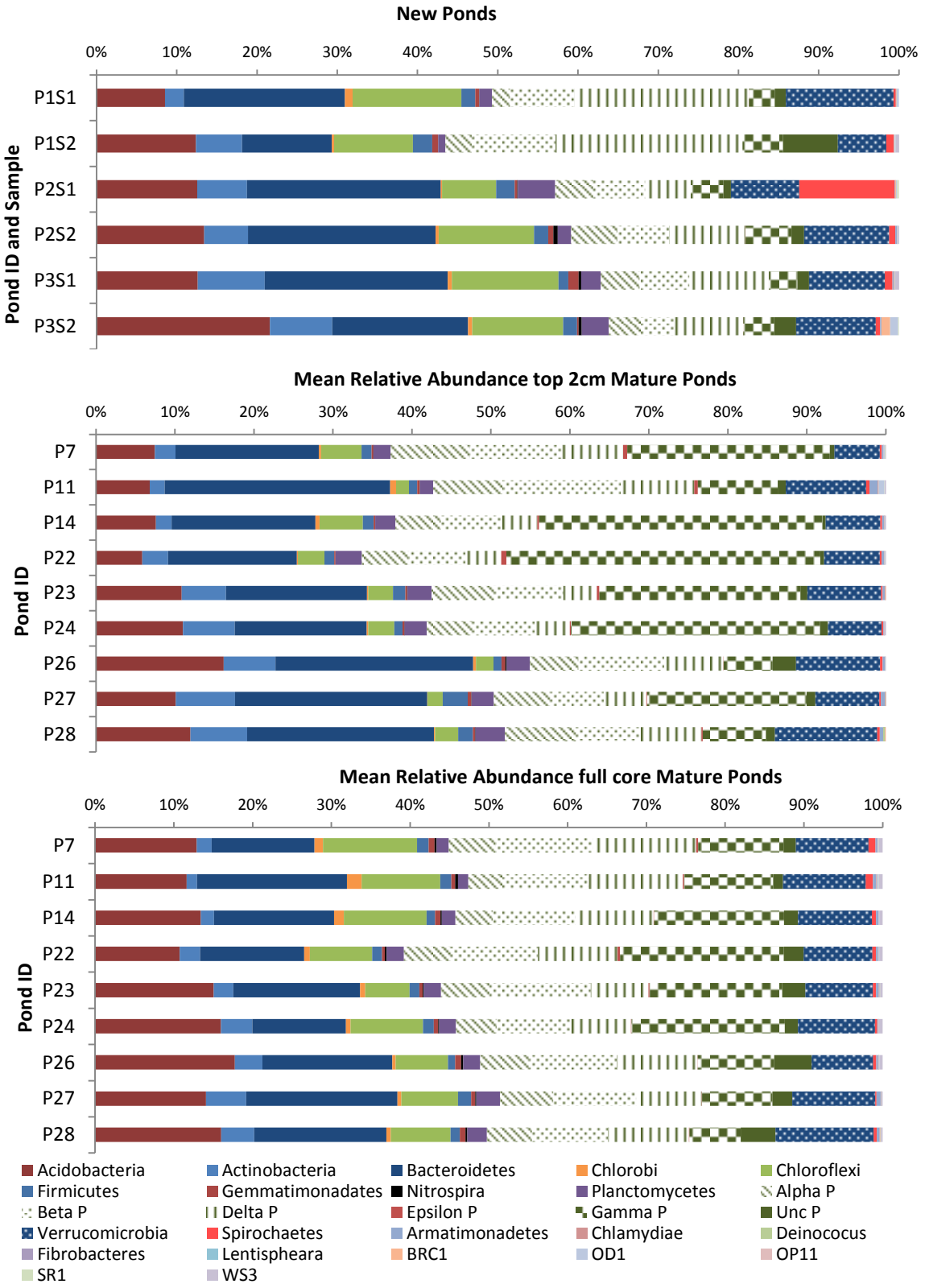


Figure VII.9: Relative Abundance of Bacterial Phylum across the new ponds (top) and for the whole core in the mature ponds (bottom).



3.3 Statistical Analysis

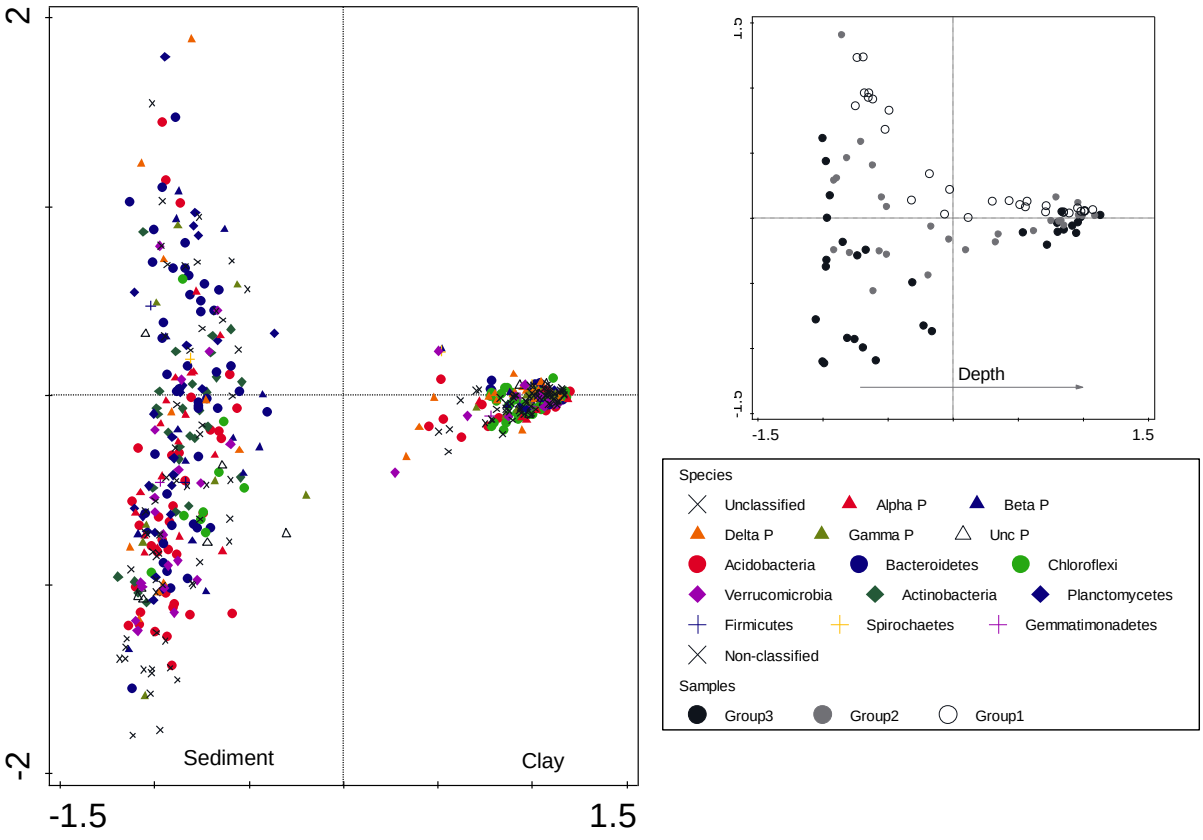
3.3.1 Bacterial Community Distribution

3.3.1i DCA analysis - mature ponds

DCA analysis was performed to establish the degree of separation between groups of ponds relative to OTU distribution. Initially PCA analysis was performed, but a strong arch affect was observed in sample distribution, indicating that a method of detrending should be applied (Ramette, 2007). Detrending was performed based on 2nd order polynomials. Percentage of explained cumulative variance was relatively low (11.32%-15.75% for axis 1 and 2), but similar to multivariate ordinations published in other studies (Ansola et al., 2014; Peralta et al., 2013).

Figure VII.10 highlights OTU and sample distribution based on the DCA analysis. Separation was most apparent along horizontal axes, which separated upper sediment layers from clay soil, indicating distinct microbial communities between the clay and sediment. Vertical axes were responsible for separating ponds by group and displayed a much wider distribution of OTUs than within lower layers, indicating a greater degree of variability in bacterial communities between ponds.

Figure VII.10: DCA Plot highlighting distribution of bacterial OTU's across mature pond sample.

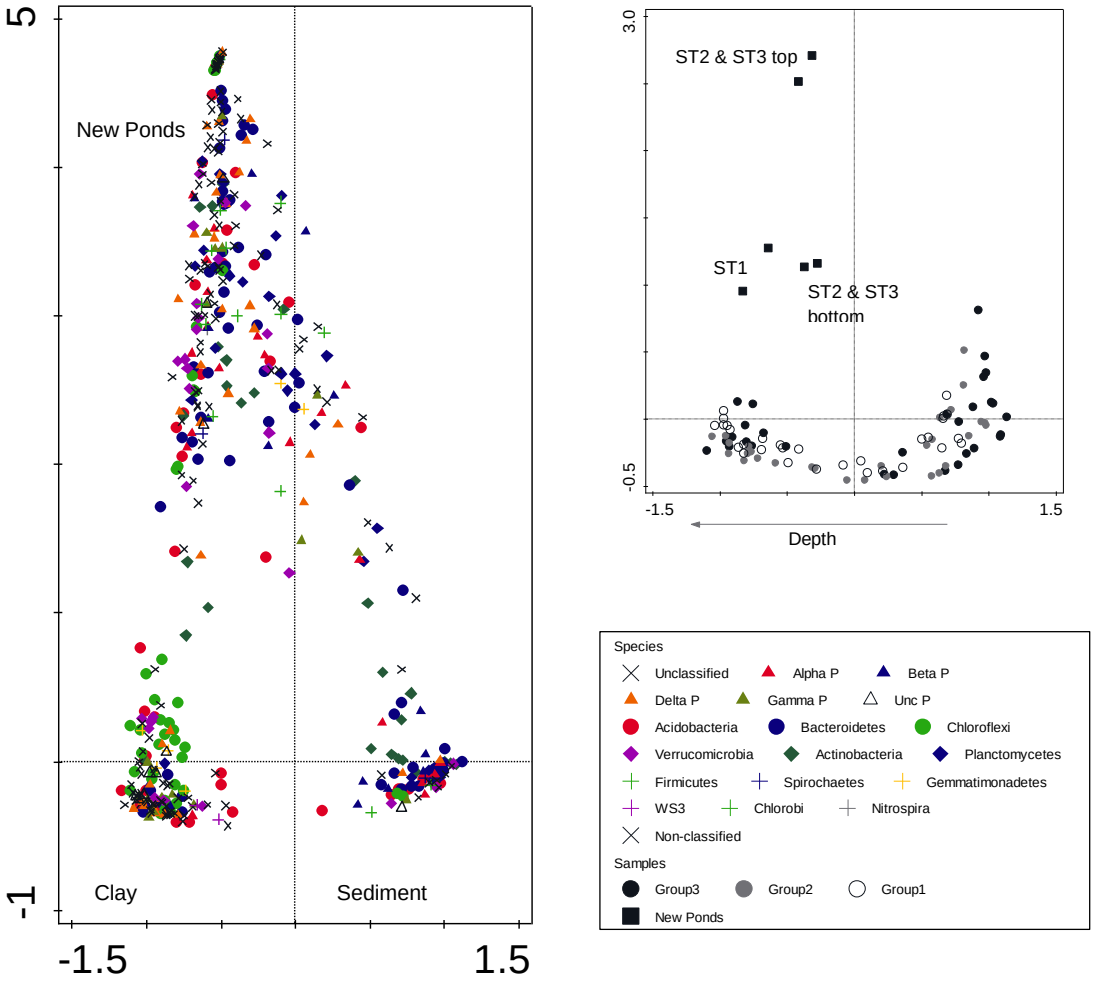


Chloroflexi, *Acidobacteria* and *Deltaproteobacteria*, which showed increasing distribution within lower layers, heavily influenced separation between sediment and clay. Variation between groups of ponds was largely driven along diversity gradient of OTUs. Diversity was higher in the group 3 clustering, displaying higher abundance of *Acidobacteria* and *Verrucomicrobia*. Group 1 and 2 ponds were less diverse in terms of OTU distribution and display higher prevalence of *Delta* and *Gammaproteobacteria*.

3.3.1ii DCA analysis mature and new ponds

DCA analysis was performed including samples from newly constructed ponds to assess sample distribution relative to mature ponds. The DCA ordination can be seen in **figure VII.12**. Explained cumulative variation was low (10.24% – 15.74%). Vertical axes separate mature ponds from new ponds; horizontal axes separate clay and soil. OTUs were more widely distributed along the vertical axis, suggesting more variability than communities within mature ponds.

Figure VII.12: DCA Plot highlighting distribution of bacterial OTU's across mature and new pond samples



OTU distribution shows distinct clustering between sediment and clay on the horizontal axis. New ponds, aligned on the vertical axis, displayed distinct difference in community composition relative to clay and sediment clusters. Points driving the largest separation on the vertical axis belonged to top sediment samples in ST2 and ST3, supporting findings in chapter V that early sediment layers are transitional, highly distinct from mature sediments.

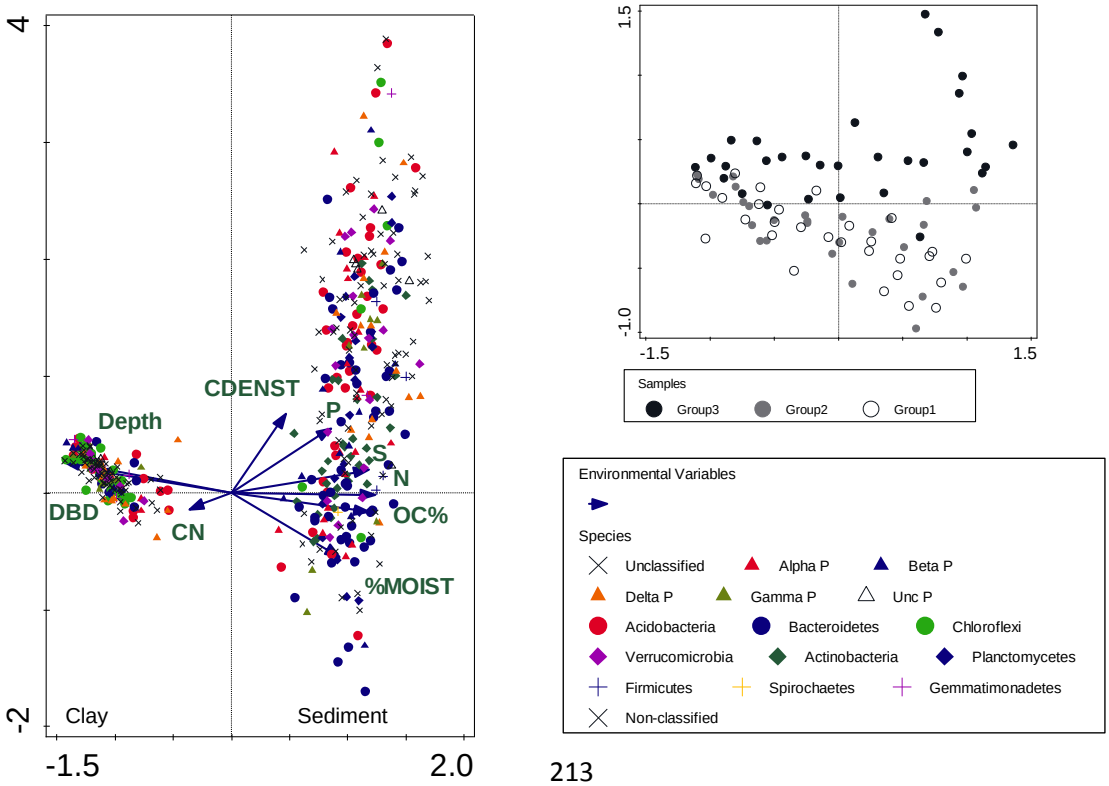
3.3.2 Impact of sediment physicochemistry on bacterial community structure

3.3.2i CCA analysis – OTU distribution and physicochemistry

Canonical Correspondence Analysis (CCA) was performed to identify the influence of physicochemical variables on the distribution of OTU's and samples. Cumulative variance explained was 42.03% - 57.14%, physicochemical variables accounted for 23.5% of explained variance. Pond groups still displayed distinct separation, largely constrained by C density and %Moisture axis. Separation between clay and sediment layers was largely constrained by the horizontal axes with depth, DBD and OC%.

Association between phylum and physicochemical variables across upper sediment layers was less apparent. Although *Acidobacteria* were generally split by C density and P, *Actinobacteria* were largely aligned between the N and S axis, and *Bacteroidetes* displayed closer association to the OC% axis.

Figure VII.11: CCA Analysis of physicochemical variables and bacterial OTU's

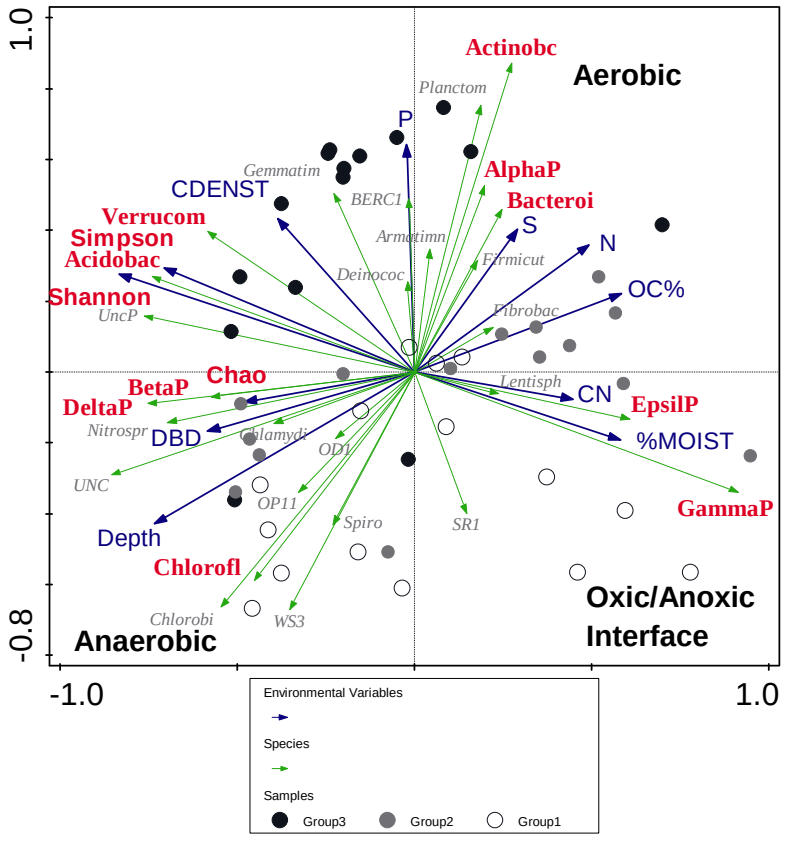


3.3.2ii PCA – Community composition and physicochemistry

PCA analysis was performed on mature pond sediment (top 5cm) samples to establish relationships between community composition and sediment physicochemistry. Figure VII.13 displays an ordination of samples and species, with physicochemical variables overlain as unconstrained variables. Explained cumulative variance across the first two axes was 76.22%. Phyla were largely split along 2 axes; depth and OC% aligned along the vertical axis, CN and %Moisture aligned along horizontal axis. Vertical distribution of samples reflects sediment depth, DBD and abundance of *Chloroflexi.* Horizontal axis split ponds by group, reflecting CN, %Moisture and to a lesser extent, C density. *Gamma* and *Epsilonproteobacteria* aligned along the right axis, against *Acidobacteria*, *Verrucomicrobia*, *Beta* and *Deltaproteobacteria* on the left.

Separation based on aforementioned phyla likely reflects redox gradients, when utilising our current understanding of these phylum (see table VII.1) *Chloroflexi*, *Deltaproteobacteria* and *Gammaproteobacteria* are associated with sulphur reduction in anaerobic conditions. *Epsilonproteobacteria* are more abundant at the oxic-anoxic interface. *Bacteroidetes* and *Actinobacteria.* were deemed to be more aerobic, alongside *Acidobacteria* and *Verrucomicrobia* which are ubiquitous in soils.

Figure VII.13: PCA Plot highlighting distribution of bacterial phylum relative abundance across top sediment sections (5cm) in mature pond samples, with physicochemical variables overlain.



3.32iii Spearmans correlation analysis

Table VII.2 displays correlation coefficient values of physicochemical variables with microbial diversity indices and relative abundance. A large number of significant relationships were observed. Diversity displayed the same pattern of significant relationships, apart from Chao index which did not display significant correlation to C density. Positive correlation of diversity indices were observed with depth, DBD and C density. Negative correlation was observed with OC%, N, S and %moisture.

Table VII.2: Correlation Coefficient values for phylum level relative abundance, diversity indices and physicochemical variables

	Depth	DBD	OC%	C Density	N	P	S	CN	% Moisture
Simpson	**.345****	**.288****	**-.361****	**.235***	**-.310****	-.050	**-.223***	-.008	**-.380****
Shannon-H	**.321****	**.277****	**-.377****	**.252***	**-.320****	-.005	**-.210***	-.093	**-.362****
Chao	**.252***	**.268***	**-.359****	.006	**-.358****	-.172	**-.261***	-.080	**-.220***
Acidobacteria	**.248***	**.343****	**-.290****	**.285****	**-.272****	-.030	-.143	-.007	**-.390****
Actinobacteria	**-.546****	**-.363****	**.413****	**.260***	**.415****	**.440****	**.431****	.058	.200
Bacteroidetes	**-.849****	**-.724****	**.736****	**.236***	**.756****	**.524****	**.759****	**-.255***	**.630****
Chloroflexi	**.777****	**.610****	**-.636****	**-.210***	**-.635****	**-.481****	**-.669****	.053	**-.512****
Alpha P	**-.622****	**-.454****	**.481****	.107	**.493****	**.275****	**.433****	.055	**.342****
Beta P	-.005	.093	-.039	**.247***	-.022	-.061	-.022	-.096	-.090
Delta P	**.615****	**.464****	**-.437****	.016	**-.407****	**-.234***	**-.464****	-.054	**-.405****
Epsilon P	**-.610****	**-.688****	**.660****	-.076	**.637****	**.284****	**.570****	-.016	**.653****
Gamma P	**-.494****	**-.355****	**.308****	**-.241***	**.265***	-.030	**.228***	.192	**.373****
Verrucomicrobia	**-.301****	**-.261***	**.280****	**.239***	**.308****	**.404****	**.329****	-.208	.202

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Depth displayed the highest number of significant relationships and the greatest degree of significance. *Acidobacteria*, *Chloroflexi* and *Deltaproteobacteria*, were positively correlated with depth. *Actinobacteria*, *Bacteriodetes*, *Verrucomicrobia, Alpha, Epsilon* and *Gammaproteobacteria*, were negatively correlated.

Species positively correlated with C density were *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Betaproteobacteria* and *Verrucomicrobia*, whereas *Chloroflexi* and *Gammaproteobacteria* displayed negative correlation. Correlations with OC%, %Moisture, N, P, S varied in significance, however, consistent patterns of positive and negative correlation were observed across these variables for individual phyla. There is a clear significant relationship between bacterial community composition and sediment physicochemistry. It is therefore important to identify significant factors controlling this.

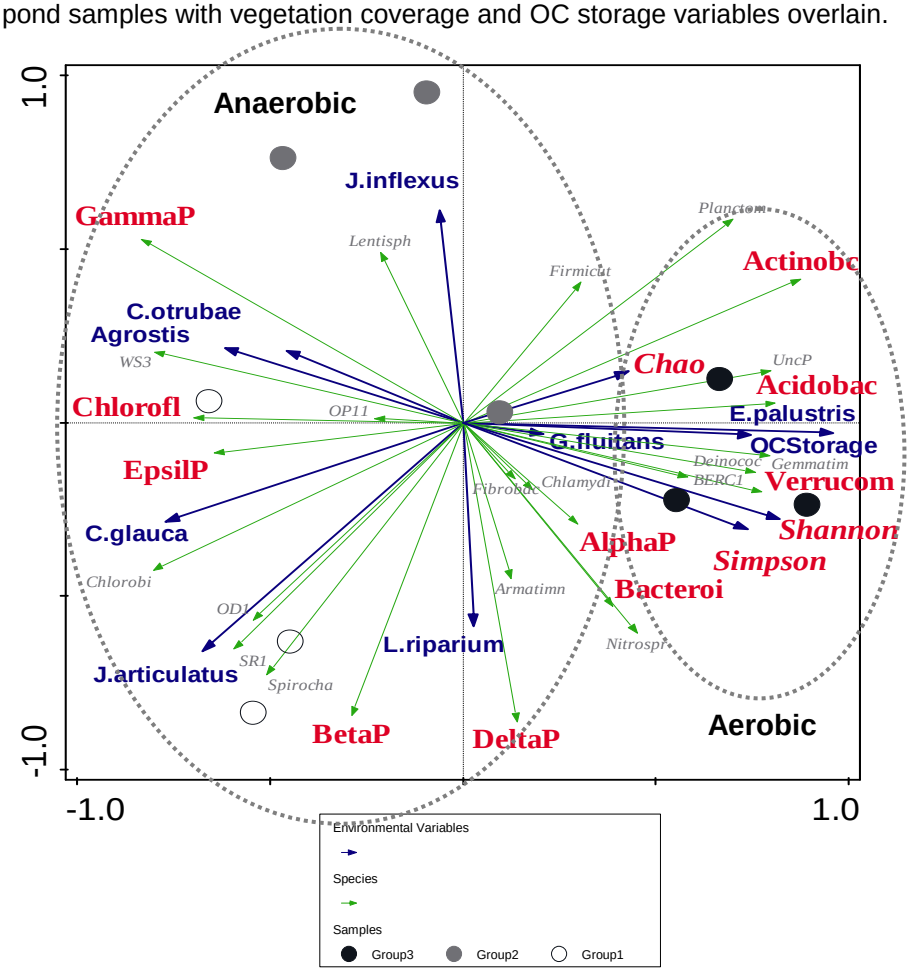
3.3.3 *Impact of vegetation coverage on bacterial community structure*

3.3.3i PCA – Community composition and vegetation coverage

PCA analysis was performed using average relative abundance of bacterial phylum across the top 5cm of sediment in ponds, to represent sediment layers. Results can be seen in figure VII.14. Percentage coverage of contemporary vegetation communities outlined in chapter II and OC storage values were overlain as unconstrained environmental variables. Explained cumulative variance was 74.78%.

Pond groups displayed distinct separation by abundance of anaerobic and aerobic bacteria. Group 1 ponds, dominated by *J.articulatus* and *C.glauca*, were closely associated with *Chloroflexi, Delta* and *Epsilonproteobacteria*. Group 2 ponds, dominated by *Agrostis*, *C.otrubae* and *J.inflexus*, were associated with *Gamma* and *Betaproteobacteria*. Group 3 ponds, displaying coverage of *G.fluitans* and *E.palustris* were associated with *Alphaproteobacteria*, *Actinobacteria*, *Acidobacteria*, *Bacteroidetes*, and *Verrucomicrobia*. Results highlight important relationships between microbial community composition and vegetation community composition.

Figure VII.14: PCA Plot highlighting distribution of bacterial phylum relative abundance across mature pond samples with vegetation coverage and OC storage variables overlain.



3.334ii Spearmans Correlation Analysis

Table VII.3 displays correlation values for average bacterial relative abundance within the top 5cm of sediment and contemporary vegetation coverage. Fewer significant correlations were observed but coefficient values were overall greater than those observed with significant relationships to physicochemical variables (Table VII.2).

Table VII.3: Correlation Coefficient values for phylum level relative abundance, diversity and vegetation coverage

	Agrostis	Carex glauca	Carex ortubae	Eleocharis palustris	Spirogyra	Glyceria fluitans	Juncus articulatus	Juncus inflexus	L.riparium
Acidobacteria	-.477	**-.804****	-.329	.075	-.010	.303	**-.775***	.137	-.297
Actinobacteria	**-.736***	**-.726***	-.639	.653	**.703***	.294	-.638	.274	-.137
Bacteroidetes	-.226	-.402	-.037	.360	.366	.183	.162	-.548	.456
Chloroflexi	.377	.630	.164	-.494	-.614	-.523	.315	.274	-.046
Alpha P	-.251	.131	-.475	**.669***	.574	.633	.111	-.411	.342
Beta P	.142	.472	.110	-.159	-.188	.220	.587	-.548	.365
Delta P	-.184	.131	-.420	.368	-.030	-.009	.468	-.548	**.730***
Epsilon P	.644	**.743***	.438	-.067	-.050	.046	.621	-.274	.137
Gamma P	.485	.481	.566	-.519	-.297	-.560	.204	.411	-.342
Verrucomicrobia	-.477	**-.726***	-.237	.427	.545	.523	-.383	-.137	-.068

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

3.3.4 ANOSIM

Analysis of similarities (ANOSIM) was used to establish significant differences in bacterial community structure between different pond groups.

Table VII.4 displays values obtained for ANOSIM analysis comparing different pond groups, and between the mature and new ponds. Results confirm there are significant differences between pond groups. This is most significant between group 1 and group 3 ($r = 0.251$, $p = 0.001$). Differences between groups 1 and 2, 2 and 3 were less significant ($p=0.007$). The degree of dissimilarity was also markedly less (groups 1 and 2 $r = 0.095$, groups 2 and 3 $r = 0.092$) in comparison to dissimilarity observed between groups 1 and 3 ($r = 0.251$).

Significant differences were also found between the mature and new ponds ($r = 0.491$, $p = 0.001$). Stronger dissimilarity was observed when analysing differences between individual pond groups and the new ponds with respective r-values of 0.557, 0.553 and 0.508 for groups 1, 2 and 3. Results suggest a greater degree of dissimilarity between mature and new ponds, compared to that observed between mature pond groups.

Table VII.4: ANOSIM statistical summary for the mature vs new ponds and between different pond groups.

Pairwise Tests		r-Value	p-Value
Mature Ponds	New Ponds	0.491	0.001
Lower Sediment Layers	Upper Sediment Layers	0.816	0.001
Group1	Group2	0.095	0.007
Group1	Group3	0.251	0.001
Group2	Group3	0.092	0.007
Group1	New Ponds	0.557	0.001
Group2	New Ponds	0.553	0.001
Group3	New Ponds	0.508	0.001

Table VII.4 displays ANOSIM values obtained from upper and lower layers, as split by cluster analysis in figure VII.5. Upper and lower layers were significantly different (p = 0.001) and the degree of dissimilarity was high (r = 0.816). The results suggest a greater degree of dissimilarity between upper and lower sediment layers than that observed across pond groups and between mature and new ponds.

3.3.5 SIMPER

Similarity percentages (SIMPER) analysis was used to identify the relative contribution of specific OTU's to differences associated between the groups being compared.

Table IX.2 (Appendices) highlights the top 30 OTUs responsible for significant difference observed between mature and new ponds. Cumulative % explanation of these OTUs was 13.8%. OTUs showing higher abundance within the mature ponds were dominated by the *Gammaproteobacteria* phylum of which *Aeromonas*, *Shewanella* and *Pseudomonas* were identified at the genus level. *Aeromonas* and *Shewanella* are both facultative anaerobes and may indicate anoxicity within the mature pond sediments as oppose to the recent sediment within the new ponds. Other OTUs included one from each of the *Bacteroidetes*, *Verrucomicrobia* and *Acidobacteria.*

OTUs showing higher abundance within the new ponds belonged to members of *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Chloroflexi* alongside *Beta*, *Delta* and *Gamma* classes of *Proteobacteria*. However the most significant OTU responsible for dissimilarity between the two types of ponds belonged to the phylum *Spirochaetes.*

Table VIX.3 (Appendices) highlights differences between sediment and clay layers, as split by the cluster analysis in figure VII.5. This also largely reflects the depth of sediment determined for individual ponds in chapter VI. A large number of OTUs were unclassified at both the phylum and class level. The *Proteobacteria* phylum was largely responsible for dissimilarity between sediment and clay, of which the *Gammaproteobacteria* class were the majority, with 9 out of the top 30 OTUs belonging to this class. *Beta* and *Delta* classes were also observed as causing dissimilarity and their functional role as sulphate reducers in anoxic conditions may highlight oxic/anoxic gradients down the core profile. It may also indicate that different processes responsible for carbon degradation and release could occur simultaneously at different depth levels down the core profile.

Tables IX.4 IX.5 and IX.6 (Appendices) highlight OTUs responsible for driving differences between different pond groups. Similar to divisions between sediment and clay layers, dissimilarity between these communities were largely attributed to the *Gammaproteobacteria* class, of which the *Aeromonas*, *Pseudomonas* and *Shewanalla* geneses featured as the most significant OTU's responsible for driving differences between microbial communities within different ponds.

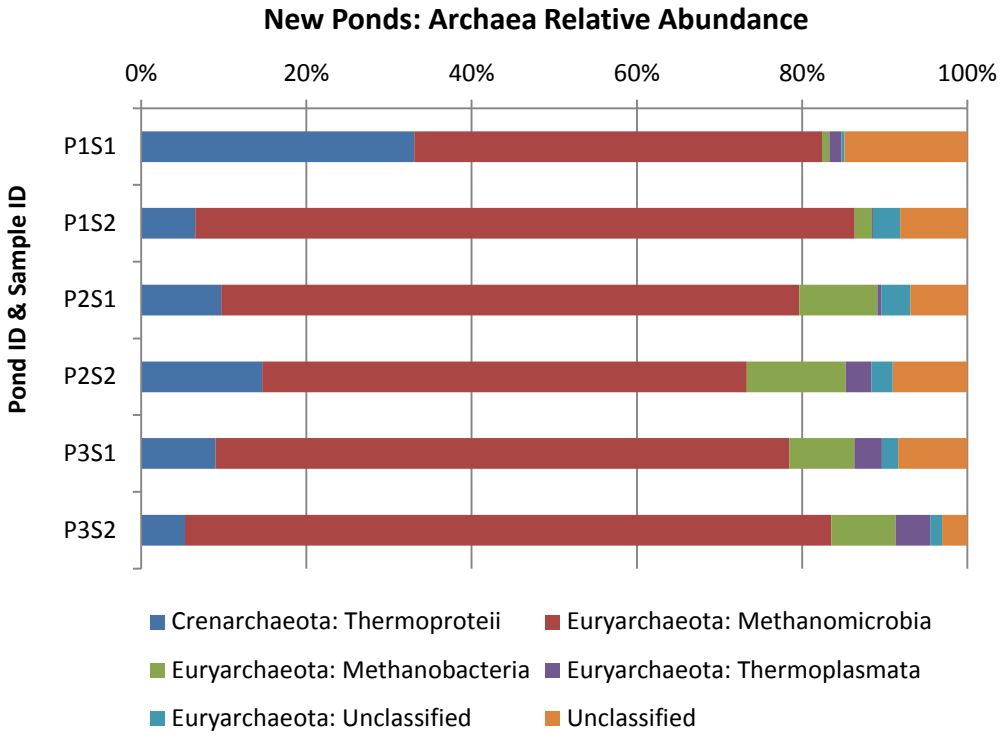
3.4 Archaea

A number of OTU's were classified as belonging to the domain Archaea. These OTU's were removed in initial analysis as the Schloss universal primers used are designed primarily for the amplification of bacterial rRNA and are not intended for amplification of Archaeal rRNA. OTU's classified as Archaea have the distinct possibility of being erroneous reads and are often treated as artefacts, so their use in data analysis must be used with a degree of caution. This limits their use in direct comparative analysis with bacterial communities.

As the nature of this chapter is exploratory, separate analysis using archaeal sequences obtained was performed. A total of 116,131 reads were classified as belonging to the domain Archaea. These reads belonged to two phylum of *Euryarchaeota* and *Crenarchaeota*, of which the former dominated reads. Three classes were observed under the *Euryarchaeota* classes *Methanomicrobia*, *Methanobacteria* and *Thermoplasmata*. Only one class of *Thermoproteii* were observed under the phylum *Crenarchaeota*.

Figure VII.15 shows relative abundance for the new ponds. *Methanomicrobia* and *Thermoproteii* dominated Archaeal classes within the new ponds. *Methanobacteria* were markedly less abundant within the new ponds in comparison to mature ponds.

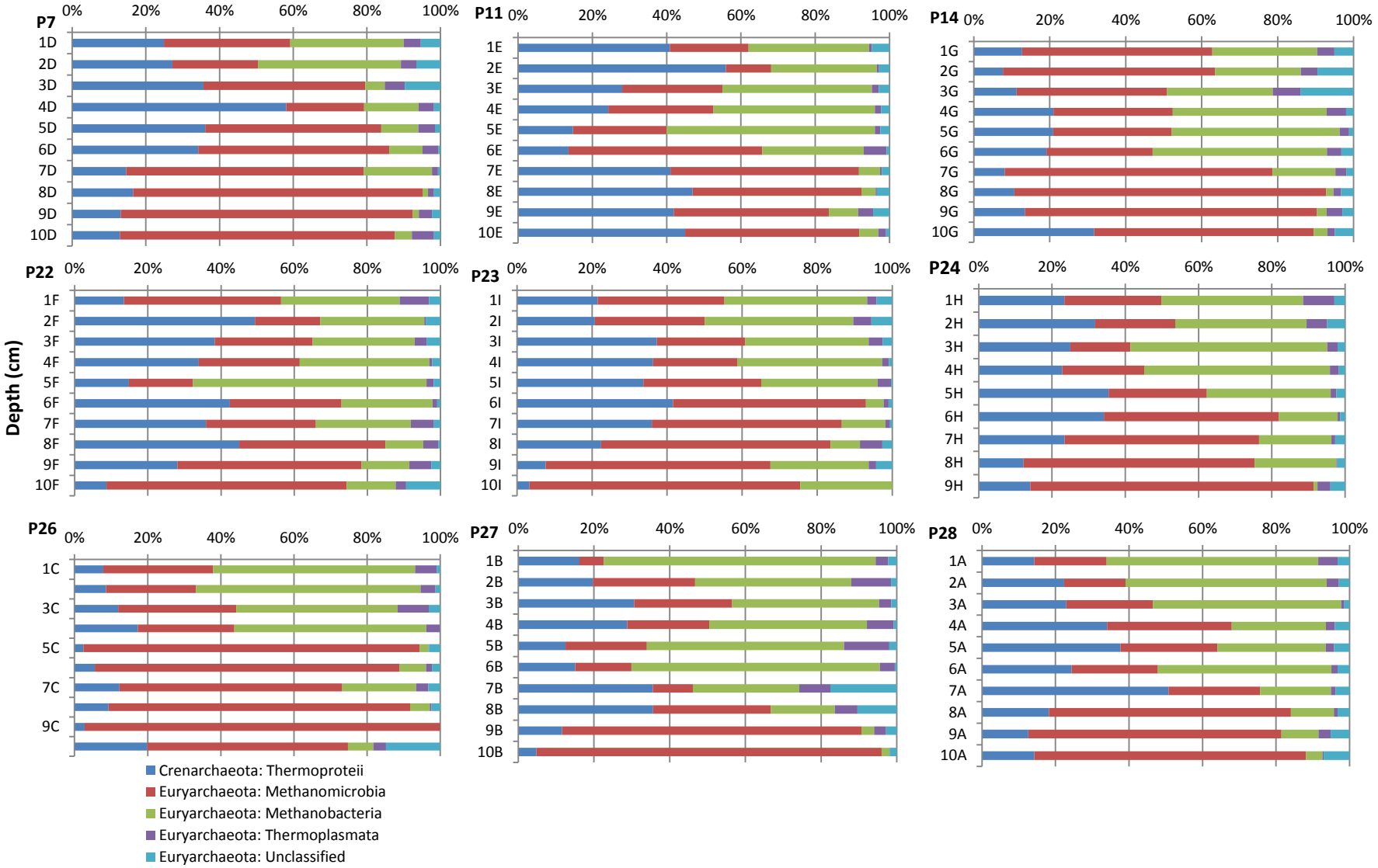
Figure VII.15: Relative Abundance of archaeal communities in the new ponds.



Relative abundance of Archaeal members at class level, in the mature ponds can be seen in figure VII.16. *Thermoplasmata*, *Methanomicobia* and *Methanobacteria* were the dominant classes observed. *Methanomicrobia* displayed generally increasing abundance down the core profile, whereas *Methanobacteria* showed a general decrease. *Thermoproteii* were generally most abundance in the upper and middle layers of the core profile. Variability between pond groups was also observed. *Thermoproteii* and *Methanomicrobia* were more abundant in pond groups 1 and 2, whereas *Methanobacteria* were generally more abundant in the group 3 ponds.

Variations between different ponds and down the core profile likely reflect relationships observed with vegetation coverage, physicochemistry and bacterial community composition.

Figure VII.16: Relative Abundance of archaea communities in the mature ponds.



4.0 Discussion

4.1 Microbial Diversity

Alpha diversity displayed a large degree of variation between ponds and down the core profile. Ponds in the lower part of the study site (Group 3) were generally more diverse than the ponds at the upper end of the site (Group 1). Group 2 ponds, apart from pond 22, were more comparable to group 3 ponds (figure VII.2). The same spatial variation was also observed across the newly constructed ponds with ST3 displaying markedly higher diversity than ponds ST2 and ST1. ST2 also displayed considerably higher diversity than ST1 (see figure VII.3).

Patterns of microbial diversity down the core profile showed considerable variation between pond groups (figure VII.1). Diversity in group 3 ponds was generally higher in the upper layers and remained fairly consistent depth, whereas in group 1 ponds, diversity was generally higher in lower sediment layers, elevated above group 3. Group 2 ponds showed less similarity throughout the group. Ponds 23 and 24 displayed higher diversity in upper layers, and pond 22 exhibited higher diversity within lower core layers, reflecting the split of this group in figure VII.5.

Variations in overall diversity between different ponds generally reflect patterns observed in sediment OC storage across the ponds (see chapter VI). It is possible that higher OC storage may result in a more diverse microbial community, or vice versa where higher microbial diversity results in an increase of OC storage. Lange et al. (2015) suggested that microbial diversity increases with OC storage. This would also be supported by elevated Shannon diversity indices in upper layers of group 3 ponds (figure VII.4).

Microbial diversity may have a number of impacts on ecosystem functions. It has been suggested that higher microbial diversity in natural wetlands indicates a greater number of ecosystem functions (Ansola et al., 2014; Peralta et al., 2013). On the other hand, it has also been speculated that lower diversity could be a result of more controlled and stable physicochemical conditions (Ansola et al., 2014).

Uncertainty around the role of microbial diversity and its subsequent effect on ecosystem function should be addressed in future studies. Understanding how this is impacted by future projected climate change, is crucial to understanding biogeochemical cycling of wetlands in a changing world.

4.2 Microbial Community Composition

4.2.1 Phylogenic groups

All sediment samples were dominated by 6 major bacterial phyla; *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Verrucomicrobia* and *Proteobacteria,* which were the most dominant phylum observed (see figure VII.6 and VII.7). To a lesser extent *Firmicutes, Planctomycetes* and *Chlorobi* were also identified as dominant phyla*.* This is similar to communities observed in other freshwater environments (Zhang et al., 2015; Ansola et al., 2014; Peralta et al., 2013; Briee et al., 2007).

Subclasses of *Proteobacteria* were dominated by *Alpha*, *Beta*, *Delta* and *Gamma* divisons, of which the *Gamma* and *Beta* classes were most abundant. *Epsilonproteobacteria* were also observed and are understood to occur in higher abundance at the oxic/anoxic interface, which may explain why their overall abundance within the dataset is low, if they are limited to these environments (Grote et al., 2011). *Epsilonproteobacteria* have been included within analysis as a marker for the niche environmental conditions promoting their increased abundance.

The dominance of *Proteobacteria* infers that the potential for biogeochemical cycling within the pond sediments is substantial. *Actinobacteria* and *Bacteroidetes* are also understood to play an active role in the degradation of both labile and recalcitrant OC sources (see table VII.1). However, the role of other dominant phyla observed such as *Acidobacteria*, *Chloroflexi* and *Verrucomicrobia* is less well understood.

4.2.2 Variation between mature ponds

Variations in microbial community composition between pond groups are clearly evident. Figure V.10 demonstrates that OTU distribution within sediments is much broader than that observed within clay soils. Variability was most apparent in upper sediment layers, suggesting pond sediments host distinct communities, largely separated by pond groups, reflecting the spatial gradient of the site. Substantially less variation was observed with increasing depth, highlighting the transition from variable sediment conditions to the uniform communities of the clay soil.

Variations between pond groups based on distributions of bacterial phyla, are convoluted but reveal generally broad patterns. Figure VII.10 suggests an increasing distribution of *Acidobacteria*, *Alphaproteobacteria,* and *Verrucomicrobia* within the group 3 ponds.

Figure VII.8 highlights that *Gammaproteobacteria* and the *Proteobacteria* phylum on the whole, are generally more abundant within group 1 and 2 ponds. To a lesser extent *Beta* and *Deltaprotebacteria* also appear more abundant within groups 1 and 2. However, abundance of the *Actinobacteria* and *Bacteroidetes* are generally higher in the group 3 ponds than the other two groups. Variations in microbial communities reflect distinct differences observed in figure VII.10 and split of samples in figure VII.5. This suggests that despite being just metres apart, the ponds host bacterial communities highly distinct from one another, increasing in dissimilarity with increasing distance along the spatial gradient of the site.

Results from the ANOSIM analysis (table VII.4) suggest that differences in bacterial communities between pond groups were significant. Greatest significance was observed between groups 1 and 3 ($r = 0.251$, $p = 0.001$), notably less than groups 1 and 2 ($r = 0.095$, $p = 0.007$), and groups 2 and 3 ($r = 0.092$, $p = 0.007$).

SIMPER analysis (tables IX.4, IX.5 and IX.6 Appendices) revealed that the majority of dissimilarity in OTU distribution and abundance between groups was largely the result of *Gammaproteobacteria* of which the genera *Aeromonas*, *Shewenella Pseudomonas* and *Yersinia* were responsible for differences between pond groups. To a lesser extent, *Beta* and *Deltaproteobacteria* and respective genera of *Thiobacillus* and *Geobacter* were also responsible for differences between pond groups. These genera are largely facultative anaerobes (table VII.5) and differences observed between ponds potentially reflect variations in sedimentary redox conditions. These genera are largely more prevalent within the group 1 and 2 ponds, highlighting differences in environmental conditions within the sediments.

4.2.3 Variation between mature and new ponds

Figure VII.11 highlights distinct bacterial communities within the new ponds in comparison to mature pond sediment and underlying clay soil. Top cm layers in ST2 and ST3, deemed to have the most characteristic sediment profile, were closely associated, supporting distinct communities from ST1 and lower layers, the latter of which showed closer association. These results would support assumptions made in chapter V that sediment within these ponds is relatively transitional, developing physicochemical profiles similar to mature sediments, but supporting distinct bacterial communities.

Variations in the top sediment layer of the new ponds were largely attributed to *Acidobacteria*, *Actinobacteria*, *Bacteroidetes* and *Proteobacteria* (see figure VII.9). Actinobacteria have the ability to withstand drought conditions, which likely accounts

for their greater abundance within the ST2 and ST3 sediments as these ponds were often subject to dry-phases (see chapter V), which are likely more extreme in comparison to mature ponds given the more exposed nature of the sediment.

Proteobacteria were more abundant within ST1. This may arise from the enhanced productivity observed in ST1, which likely results in a large degree of labile carbon deposition. Although, this pond did not show a characteristic sediment profile (see chapter V).It is possible that this initial layer may have been highly flocculent and was perhaps not captured during the coring process. The finer resolution of DNA sampling (~0.25 g) may have captured remnants of this layer, whilst dissecting layers at 1cm for physicochemical analysis may have limitations for such a fine transitional layer.

Overall, *Proteobacteria* were more abundant in mature ponds (Figure VII.9). *Gammaproteobacteria* in particular showed a much greater abundance in mature ponds, suggesting that *Gammaproteobacteria* increase in abundance as sediments develop. *Epsilonproteobacteria* were largely absent in the new ponds, this class of *Proteobacteria* are often found at the oxic-anoxic interface within sediments and may suggest a lack of this characteristic within the newly accumulated sediment (~3yrs).

Table VII.5: Overview of *Proteobacteria* genera responsible for driving dissimilarity down the core profile and between different types of pond as outlined by SIMPER analysis.

Phylum	Genus	Overview	Reference
Beta	Thiobacillus	Facultative chemolithoautotroph Use sulphur as an energy source and oxidise carbon in the process	Childers, S (2002)
Delta	Geobacter	Found in anaerobic conditions Involved in anaerobic respiration of organic compounds including iron and petroleum compounds	Heider, J & R. Rabus (2008) Loveley et al., (1987)
	(Family)Desulfobacteraceae	Facultative Anaerobe Reduce sulphates to sulphides as an energy source	Garrity et al., (2005)
Gamma	Aeromonas	Facultative anaerobe Ubiquitous in freshwater	Graf, J (2015)
	Pseudomonas	Great deal of metabolic diversity Most species aerobic some anaerobic	Maidgan, M & J. Martinko (2005)
	Shewanella	Facultative Anaerobe Ability to reduce iron and manganese	Fredrickson et al., (1998)
	Yersinia	Facultative Anaerobes Proliferate at temperatures as low as (1-4°)	Tan et al., (2015)

ANOSIM analysis suggest that dissimilarity between the mature and new ponds was high (r = 0.491, p = 0.001) and was greater than that observed between pond groups. SIMPER analysis (table IX.2 Appendices) suggests that *Gammaproteobacteria (Genera: Aeromonas, Shewenanella* and *Pseudomonas)* were responsible for driving a large proportion of dissimilarity between the mature and new ponds. These genera are all largely associated with anoxic conditions (table VII.5) and were more abundant within the mature ponds, indicating an absence of anaerobic conditions in the new ponds. *Bacteriodetes* were also largely responsible for dissimilarity observed between the mature and new ponds.

Marked differences in abundance of the *Proteobacteria* phylum, which are associated with biogeochemical cycling of important elements, carbon in particular, might suggest that perhaps these processes are less substantial within the newer ponds than the older ponds and would reflect associated levels of OC storage and burial.

4.2.4 Variation down the core profile

Cluster analysis (figure VII.5) identified a general separation of upper and lower sediment core layers. Interestingly this split somewhat reflects depth layers identified in chapter VI, indicating a clear split in microbial community structure between sediment and clay based layers. This split is further evident in Figures VII.10 and VII.11, which highlight distinct clusters of OTUs along the horizontal axis, reflecting differences between sediment and clay. Clay layers displayed markedly less variation in regards to OTU distribution and bacterial community.

Figure VII.8 also suggests that the abundance of *Chloroflexi*, *Beta* and *Deltaproteobacteria* increases with depth, whilst *Bacteroidetes and Actinobacteria* decrease. Class level distribution within the *Chloroflexi* phylum was largely dominated by *Anearolineae*, which have been identified in wetland environments and methanogenic wastewater treatment systems (Matsuura, 2015; Ansola et al., 2014). *Chloroflexi* favour anoxic environments, which likely explains their increasing abundance down the core profile. *Beta* and *Deltaproteobacteria* are also facultative anaerobes largely involved in sulphur reduction processes. Observed patterns down the core profile likely reflect redox and environmental conditions down the core profile.

ANOSIM analysis suggests that dissimilarity between upper and lower sediment layers is more significant (r = 0.816, p = 0.001) than any of the other permutations analysed as part of this study, which likely reflects the contrasting physicochemistry of these substrates. SIMPER analysis (table IX.3 Appendices) suggests that this dissimilarity is driven by *Gammaproteobacteria (*Genera *Aeromonas*, *Shewenella Pseudomonas* and

Yersinia), which were generally more abundant in upper sediment layers, *Deltaproteobacteria (Genera*: *Geobacte*r and Family: *Desulfobacteraceae*) and *Betaproteobacteria* (Genera: *Thiobacillus*) were also observed as driving dissimilarity between sediment and clay layers. These genera are largely associated with anaerobic environments see table VII.5 and likely reflect anoxia within lower clay layers.

4.2.5 Relationship to physicochemical variables

Diversity patterns within the core profile of individual ponds potentially reflect physicochemical gradients. All three diversity indices used were significantlly correlated to depth, DBD, OC%, N, S and %moisture (see table VII.2). Diversity was positively correlated to depth and DBD. Simpson and Shannon indices were also positively correlated to C density, which supports the Lange et al. (2015) proposal that microbial diversity increases with OC storage. Diversity was negatively correlated to OC%, N, S and %moisture. Despite displaying highly significant p-values (majority <0.01) the strength of correlated variables was not particularly high with no coefficient values exceeding ± 0.390. Mean diversity values (figures VII.4) follow a similar pattern to that observed in OC storage between the ponds (Chapter VI). Increasing diversity would further support findings from Lange et al. (2015), which demonstrate increasing OC storage with increasing microbial diversity.

The impact of physicochemical variables on microbial community structure is also highlighted in figure VII.12. C density and %moisture accounts for the separation of pond groups in upper sediment layers. Figure VII.13 highlights association between the relative abundance of bacterial phylum with physicochemical variables. Horizontal axis split pond groups by C density and %Moisture. Vertical axis largely split samples by depth and OC%. Depth displayed the largest number of significant relationships, both positive and negative (see table VII.2).

Depth shapes physicochemical and down the core profile, playing a significant role in structuring microbial community composition. *Chloroflexi* were closely associated with depth and increasing in abundance down sediment core profiles (figure VII.8), a result of their preference for anaerobic conditions (Zhang et al., 2015). *Acidobacteria*, *Verrucomicrobia*, and *Deltaproteobacteria* also displayed significant positive correlation with depth. Abundance of *Beta* and *Deltaproteobacteria*, involved in sulphur reduction, would infer anoxic conditions (VII.1 and VII.10), whilst *Acidobacteria* and *Verrucomicrobia* are ubiquitous displaying relatively consistent patterns with depth (figure VII.8). *Actinobacteria*, *Bacteroidetes*, *Gamma* and *Epsilonproteobacteria* displayed significant negative correlation with depth indicating a preference for upper

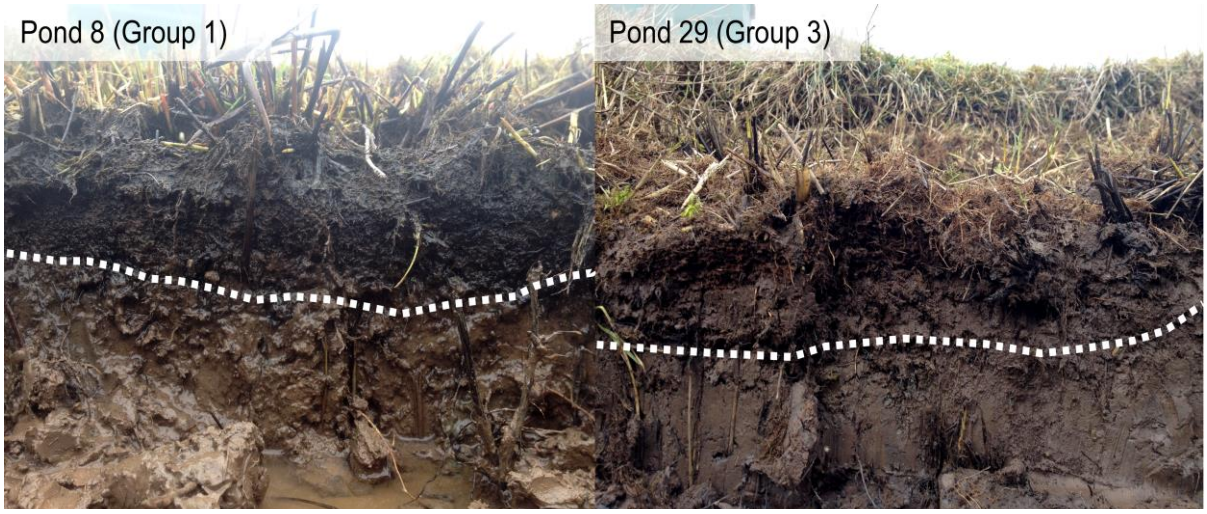
sediment layers. These phyla may therefore anoxic upper layers within the sediments, perhaps preferring younger or more labile OC sources.

Deeper sediment layer samples in all ponds migrate towards anaerobic associated communities of *Chloroflexi*, *Beta* and *Deltaproteobacteria, Acidobacteria* and *Verrucomicrobia.* Upper sediment layers display the greatest degree of separation. Group 1 and 2 ponds, higher in %moisture and OC% are more closely associated with *Gammaproteobacteria*, which are largely facultative anaerobes, and *Epsilonproteobacteria,* which are more abundant at oxic-anoxic interfaces. Diversity was lower across these groups and was negatively correlated with %moisture.

Horizontal axes in figure VII.13 largely split samples by pond groups, based on CN, %Moisture and C density. Group 1 and 2 ponds, displaying higher %moisture and C:N, were more closely associated with *Gamma* and *Epsilonproteobacteria.* Group 3 ponds, displaying higher C density, were more closely associated with *Actinobacteria* and *Bacteroidetes*. Given the nature of bacterial diversity and phylum split along these axis it is likely that distribution of sample points reflects changing redox conditions, highlighted in figure VII.13, and community dissimilarity observed in figure VII.5.

Group 3 ponds were separated distinctly from groups 1 and 2. Overall diversity and abundance of *Bacteroidetes* and *Actinobacteria* was greater, indicating that environmental conditions within these ponds are distinct from groups 1 and 2, likely more oxic (figure VII.13). Visual observations in sediments from these ponds would further support the proposition that group 3 ponds are more oxic and that wetter conditions in group 1 and 2 ponds, create anoxic conditions, restricting overall diversity, increasing in a greater abundance of anaerobic communities (figure VII.17).

Figure VII.17: Visual differences in sediment characteristics between pond groups 1 and 3.



Results suggest physicochemistry plays a significant role in shaping microbial community structure. Different physicochemical properties between different types of ponds and down the core profile, explain variations observed in microbial community composition. It also possible that rates of biogeochemical processes within the sediment could also be impacted (Hahn, 2006). Depth was responsible for driving changes in down core anoxia and abundance of soil based microbes. C density, %Moisture and OC% were significant factors influencing microbial community composition in upper layers, reflecting distinct environmental gradients in sediments between pond groups, shaping significantly different bacterial communities.

Determining whether microbial communities shape physicochemical conditions within the sediment or whether sediment physicochemistry shapes microbial community composition is difficult. It is likely the two are interlinked, responding dynamically to changes in environmental conditions and OM inputs. The following section will discuss the impact of vegetation community coverage, known to significantly affect sediment physicochemistry and microbial community composition (figure VII.14).

4.2.6 Relationship to vegetation coverage

A relatively small number of statistically significant relationships were observed between vegetation coverage and microbial relative abundance (see table VII.3). However, coefficient values were generally higher than those observed in relationships to physicochemical variables suggesting that vegetation may play a more significant influence than sediment physicochemistry. The majority of significant relationships were negatively correlated, particularly to *Agrostis*, *Carex glauca* and *Carex ortubae* . Positive correlations observed include that of *Actinobacteria* with *Spirogyra*, the more labile material from the algae may promote an increased abundance of these phyla. *Deltaproteobacteria* were positively correlated with *L.riparium* potentially due to the often anoxic environments created by the thick moss swards. *Epsilonproteobacteria* were positively correlated to *C.glauca*, as this phylum is generally most abundant at the oxic-anoxic interface it is possible that this species and associated root activity, may promote such conditions within sediments.

Separation between pond groups and bacterial phylum based on vegetation coverage are displayed in figure VII.14. Group 1 ponds dominated by *Juncus articulatus* and *Carex glauca* were closely associated with *Deltaproteobacteria* and *Epsilonproteobacteria*. Group 2 ponds dominated by *Agrostis*, *Carex ortubae* and *Juncus inflexus* were closely associated with *Gammaproteobacteria* and *Betaproteobacteria.* Group 3 ponds, dominated by *Eleocharis palustris* and *Glyceria*

fluitans were closely associated with increasing abundance of *Acidobacteria*, *Verrucomicrobia*, *Actinobacteria* and *Bacteroidetes*. Results further indicate higher abundance of ubiquitous soil based and drought resistant bacteria in group 3 ponds and more anaerobic communities in groups 1 and 2.

J.articulatus were negatively correlated with *Acidobacteria,* perhaps restricting the abundance of this primarily soil based bacteri*a,* due to the formation of more characteristically aquatic sediments, higher in %moisture*. Juncus* species are known to promote carbon fluxes out of peatland systems, through the release of labile litter and root exudates. This may account for its association with OC% in figure VI.11 and negative correlation to OC storage in chapter VI, which would support this statement. Labile litter material and root exudates, stimulate microbial activity via the rhizospheric priming effect (Lange et al., 2015). Evidence suggests released labile carbon stimulates microbial communities, increasing their activitiy in the surrounding soil promoting increased rates of OM degradation, increased microbial activity in combination with wetter conditions, promotes the anoxic conditions observed in figure VII.17 (Lange et al., 2015; Shackle et al., 2000).

Groups 1 and 2 displayed strong association with *Juncus* species and were closely associated with bacterial phylum associated with anoxic environments and sulphate reduction processes. Hydrology played a deterministic effect in shaping vegetation communities within the ponds. The establishment of Juncus and Carex species in the wetter ponds would appear to have resulted in the development of distinct physicochemical differences in the group 1 and 2 ponds, in relation to group 3. Chapter VI highlighted that *L.riparium* was most significant in promoting higher OC storage in ponds and became established in the majority of ponds from around 2006. These results would indicate that the establishment of further vegetation communities within the developing sediment layer, results in the observed physicochemical differences seen in figure VII.17 and environmental conditions, which drive variations in microbial community composition.

Results suggest vegetation plays significant role in shaping microbial community structure, potentially more significant than that observed with physicochemical variables. Wetter conditions have promoted the establishment of *Juncus* vegetation, forming wetter sediments, rich in OC% but restricted in microbial diversity to facultative anaerobes. This has ultimately led to significantly lower levels of OC storage. Drier conditions have facilitated the establishment of more marginal aquatic vegetation of *E.palustris* and *G.fluitans,* dry-phases also facilitate the establishment of strictly terrestrial species, such as *R.crispus* and result in an increased abundance of drought

resistant *Actinobacteria.* These conditions have resulted in higher levels of bacterial diversity through less restrictive conditions, ultimately resulting in larger amounts of OC storage. Clearly a complex interplay exists between hydrology, vegetation coverage and sediment physicochemical development. These factors create distinct environmental conditions, shaping microbial community composition and no doubt functions, ultimately governing OC storage and burial within the ponds.

4.2.7 Archaea

Archaea were included as an exploratory component within this chapter given limitations within the methods used. It is also important to note that phylogenetic information on archaeal community in freshwater lake sediment is still limited (Zhang et al., 2015). Studies on lakes have highlighted contrasting dominance of the *Crenarcaeota* and *Euryarchaeota* phylum (Zhang et al., 2015).

Archaeal OTU's comprised a relatively small component of the overall data set in comparison to Bacterial OTUs. *Crenarchaeota* and *Euryarchaeota* phyla were identified, of which *Euryarchaeota* dominated overall community structure in the majority of samples (see figure VII.15). Within the *Crenarchaeota* phylum only one class was identified and designated to *Thermoproteii,* whilst three classes were identified within the *Euryarcaeota* class; *Methanomicrobia, Methanobacteria* and *Thermoplasmata*. Evidence suggests *Thermoplasmata*, *Methanobacteria* and *Methanomicrobia* are methanogenic (Zhang et al. et al., 2015; Lino et al., 2013).

Similar patterns in the relative abundance of Archaeal classes were observed between pond groups and down the core profile. However, unclassified Archaeal samples were more abundant within the group 1 ponds, particularly in the lower depth layers. *Crenarchaeota* displayed fairly idiosyncratic variations down the core profile and between individual ponds. The *Methanobacteria* and *Methanomicrobia* classes of *Euryarchaeota* displayed the most consistency down the core profile and between pond groups. *Methanomicrobia* displayed a general increase whereas *Methanobacteria* showed a relative decrease down the core profile in the majority of ponds. It is possible that these changes reflect variations in redox conditions down the core profile or potentially a preference for newer, or older, carbon sources.

Figure VII.16 highlights Archaeal relative abundance for the newly constructed ponds. Abundance of *Methanobacteria* is substantially less than that observed within the mature ponds, potential indicating environmental conditions or that this class of Archaea prefer more mature carbon rich sediments. As a methanogenic species this could impact the release of CH_4 from the ponds and so differences in its relative

abundance between old and new systems may also reflect differences in the fluxes and emissions of CH_4 across successional stages of the pond.

4.3 Implications for Carbon Cycling and Storage

This study has identified dominant microbial communities present within the Hauxley ponds. A number of microbial species involved in biogeochemical cycling of carbon have been identified within the ponds, particularly the bacterial phylum *Proteobacteria* and methanogenic classes of Archaea. Bacterial phylum such as *Actinobacteria* and *Bacteroidetes* were also found in relatively high abundance and are reportedly involved in the breakdown of organic substances of labile and recalcitrant composition (Zhang et al. et al., 2015; Song et al., 2012).

Variations between different ponds were largely attributed to abundance of *Proteobacteria*. It is established that the predominance of *Proteobacteria* infers active involvement in functioning and processes of freshwater sediments (Zhang et al. et al., 2015; Peralta et al., 2013; Song et al., 2012). *Proteobacteria* were markedly less abundant within the new pond sediments. *Gamma, Delta and Epsilonproteobacteria* were more abundant in sediments storing less OC, whilst sediments storing more OC had a higher abundance of *Actinobacteria* and *Bacteroidetes*.

Dissimilarity between different ponds was largely driven by *Gammaproteobacteria. Gammaproteobacteria* display a large degree of metabolic diversity in relation to carbon and sulphur cycling in coastal sediments (Dyksma et al., 2016). It is therefore possible that they play a similar role in the ponds at Hauxley. Genera of *Aeromonas*, *Pseudomonas*, *Shewanella* and *Yersinia,* were responsible for driving significant variations between pond groups. Genera of *Geobacter* within the *Deltaproteobacteria* phylum and *Thiobacillus* within the *Betaproteobacteria* phylum were also responsible for driving significant dissimilarity between pond groups. Aforementioned genera are predominantly facultative anaerobes, involved in sulphur reduction processes. Alongside the abundance of *Epsilonproteobacteria*, which occur in high abundance at oxic-anoxic sediment interfaces, suggests different redox conditions across the ponds. The majority of these phyla displayed significant relationships to OC% and %moisture, favouring wetter and OC rich sediments. Ponds exhibiting these conditions also displayed less overall microbial diversity, likely the result of anaerobic conditions restricting activity of obligate aerobic bacteria.

Actinobacteria and *Bacteroidetes* were more abundant in group 3 ponds, favouring the drier, and more C dense sediments. These sediments were substantially more diverse than other pond groups, supporting emerging research that OC storage increases with

microbial diversity (Lange et al., 2015). Higher diversity within wetland sediments has also been attributed to increased ecosystem functions (Ansola et al., 2014). *Actinobacteria* play an important role in recycling of OM, contributing to carbon remineralisation. The ability of this species to withstand drought (Ansola et al. et al., 2014), likely accounts for the higher abundance observed within group 3. *Bacteroidetes* play a substantial role in processing complex molecules within freshwater sediments (Zhang et al., 2015) and may favour recalcitrant OC sources within these ponds.

Methanogenic communities of *Thermoplasmata*, *Methanobacteria* and *Methanomicrobia* dominated Archaeal communities identified within the ponds. Distinct variability was observed between different pond groups, particularly a greater abundance of *Methanobacteria* in group 3 ponds compared to group 1, which displayed a larger abundance of *Thermoproteii* and *Methanomicrobia*. This likely reflects variations in redox conditions identified between ponds. Regardless, there is a substantial capacity for these systems to operate as sources of CH_4 to the atmosphere (Zhang. et al., 2015). Flux rates taken from the Hauxley ponds did not identify any CH_4, likely a result of oxidation of the sediment during the drying phase.

In the context of Hahn's (2006) study, reported differences in microbial community structure, particularly *Proteobacteria* (Strickland et al., 2009), between different ponds and down the core profile may result in different rates of biogeochemical processing. Research on flux rates of C from the Hauxley ponds suggests that the ponds can switch from a net intake (-461 ± 1490 mg m^{-2} d^{-1}), to a net source (3792 ± 2755 mg m^{-2} d^{-1}) of carbon, over the course of a transitional drying period (Gilbert et al., 2016). It is possible that spatial variations observed in between ponds in this study, reflect differences in microbial community composition.

Variations in bacterial community structure between ponds was attributed to a complex interplay between hydrology, vegetation community and sediment physicochemical development, the combination of the three shaping distinct environmental conditions, ultimately leading to substantial variations in OC storage and burial. However, little is known about how the phylum and species composition of archaea and bacteria affect carbon fluxes from the ponds despite it being established that they play a substantial role in freshwater sediment environments, undoubtedly contributing to CO_2 and CH_4 fluxes from the system. Research elucidating microbial community response under different environmental conditions, such as those proposed under future climate regimes is vital if we are to understand ecosystem functioning and net carbon balances of these small water bodies. This may be a crucial component governing their acceptance as carbon mitigation options under polices such as CAP.

5.0 Conclusion

This chapter has provided insights into microbial community structure, in small constructed ponds identifying significant variability in microbial diversity and community structure between adjacent ponds, alongside factors driving this. Significant differences were observed between mature pond sediments and newly constructed ponds still developing an initial sediment layer. As ponds mature and undergo succession, it is likely microbial community structure responds or even drives this process through sediment development, similar to the successful development of soils in treatment wetlands (Ansola et al., 2014).

Significant differences in microbial community composition were associated with the abundance of facultative anaerobes belonging to *Beta*, *Delta* and *Gammproteobacteria*, indicating distinct environmental redox conditions between different ponds. Vegetation coverage and sediment physicochemistry were found to have a significant influence on bacterial community composition. Wetter sediments with high OC%, were less diverse and supported communities of facultative anaerobes, but ultimately stored less OC. Drier sediments with a higher OC density, were more diverse, stored more carbon and supported a lower abundance of *Proteobacteria*. Microbial community structure in the ponds is governed by complex interactions between hydrology, its effect on vegetation coverage, physicochemical development of the sediment and the distinct environments, which these factors create. Cumulatively these factors ultimately determine OC storage and burial within the ponds.

Differences in microbial community composition indicate the potential for differences in biogeochemical cycling and process rates, in systems just metres apart. This may explain spatial variations observed in C flux rates between the ponds in a recent study (Gilbert et al., 2016). Further research into the role of bacterial and archaeal community composition in relation to flux rates and environmental conditions (i.e. pond hydro-period) should be carried out in an attempt to comprehensively elucidate the role of these microbes in carbon cycling. Understanding the functionality of microbial communities under different environmental conditions, such as hydro-period and in relation to vegetation coverage can also inform the construction and strategic engineering of ponds for targeted functionality in regards to OC burial and reduced emissions.

Key Findings:

1. Identify dominant microbial communities present within the ponds

- All sediment samples were dominated by 6 major bacterial phylogenic groups; *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Proteobacteria* and *Verrucomicrobia.* Results are similar to those observed in other studies focusing on wetland environments.

2. Assess variations in microbial diversity and community structure between different ponds and down the core profile

- *Proteobacteria*, are generally more abundant within ponds that store less OC (Groups 1 and 2). *Actinobacteria* and *Bacteroidetes* were more abundant in ponds storing more OC (Group 3).

- *Chloroflex*i and *Firmicutes* were generally more abundant within the newly constructed ponds whilst the *Proteobacteria* phylum, in particular the *Gamma* sub class, was markedly less abundant.

- Down core variations were largely associated with the *Chloroflexi* phylum, which increased in abundance, alongside *Deltaproteobacteria*. *Gammaproteobacteria*, *Actinobacteria* and *Bacteroidetes* decreased with depth.

- Variations between ponds and downcore were largely driven by facultative anaerobic genera of *Aeromonas, Pseudomonas, Shewanella and Yersinia,* in the *Gammaproteobacteria* class, and *Geobacter* and *Thiobacillus* in the *Delta* and *Betaproteobacteria* classes.

3. Identify relationships between microbial community composition, sediment physicochemistry and vegetation coverage

- Ponds dominated by *Juncus* species with wet, looser and high OC% sediments (Groups 1 and 2) were less diverse and had a higher abundance of *Gamma*, *Beta* and *Deltaproteobacteria* and displayed a greater abundance of anaerobic species within these respective classes.

- Ponds storing more OC with denser sediments, had a greater abundance of aerobic and drought resistant bacteria and were more diverse.

- The hydrology and vegetation coverage across the site created distinct environmental gradients between pond groups promoting differences in microbial community composition.

4. Explore the implications for OC storage and carbon cycling

- Differences in microbial community structure between different ponds and down the core profile may result in different rates of biogeochemical processing according to Hahn (2006).

- Differences in microbial community structure between ponds were largely attributed to genera of facultative anaerobes, *Aeromonas* in particular, that are well established as playing a functional role in the remineralisation of carbon.

Chapter VIII

Final Reflections

1.0 Overview

This thesis has investigated OC storage and burial across a set of small constructed experimental ponds. The fully documented ecological history of the ponds since their construction ~20 years ago has facilitated the novel approaches used in this study and has provided unique insights into factors driving OC burial in small constructed ponds.

Novel data on OC burial rates in small constructed ponds is provided, enabled by the known construction date of the ponds. The exhumation of entire ponds and statistical determination of sediment depth layers has provided invaluable insights into the accuracy of sediment cores in producing whole pond OC storage values, providing the first ever baseline of accuracy for sediment core extrapolations. The comprehensive recorded vegetation history of the ponds has also enabled analyses of the intrinsic association between vegetation community succession and OC storage. Three new ponds were also constructed and monitored at fortnightly intervals, providing unique data on physicochemistry, hydro-period, and perhaps most importantly OC burial over this early stage of pond succession.

This thesis has provided new data for an understudied ecosystem, small constructed ponds, and has provided evidence to support their potentially important role in the global carbon cycle, alongside their potential as an ecologically and economically efficient tool in the construction of sustainable landscapes.

1.1 Carbon Storage

Results from chapter IV provide novel insights into the accuracy of sediment coring methods and OC burial in comparison to the surrounding soil. Chapters V and VI provide a more comprehensive analysis of OC storage in both mature (~20 years) and newly constructed (~4 years) old ponds, providing insights into OC storage and burial across different stages of pond succession. OC storage varied considerable between pond groups with groups 1, 2 and 3 displaying values of 1618 ± 212, 1996 ± 261 and 4077 ± 534 g OC respectively. One of the key findings from the newly constructed ponds was that OC storage over this stage of succession is negligible, with characterised sediment layers showing little OC storage (24.21-57.24 g OC).

1.2 Carbon Burial Rates

Burial rates have usually been produced using bathymetric or sediment trap surveys, which are subject to a large degree of variability and error. This study has utilised OC storage estimates and the known construction age of the ponds to give an accurate

representation of OC buried over a known timescale, a rarity in this field of research. Burial rates ranged from 67 ± 8.7 to 212 ± 27.75 g OC m^{-2} yr^{-1} and were on average 122 ± 15.97 g OC m^{-2} yr^{-1}. These burial rates are significant in themselves providing new data on OC accumulation in small constructed ponds, which are underrepresented in studies on carbon cycling, allowing comparison to their better studied larger counterparts. Augmented with data from chapter V, which has quantified burial rates for newly constructed ponds over a period of 3yrs, it is apparent that OC storage and burial across this phase is negligible (8.07 ± 19.08 g OC m^{-2} yr^{-1}).

Factoring for negligible burial across the first three years of succession OC burial rates may actually be in the region of 142 ± 18.59 g OC m^{-2} yr^{-1}. This study has also identified that the establishment of species, such as *J.articulatus,* can negatively affect OC storage, whilst *L.riparium* and *G.fluitans* promote enhanced OC burial. Mature ponds displaying earlier establishment of these species stored substantially more carbon than other ponds retaining more aquatic based plant species. The newly constructed ponds also demonstrated that the early establishment of these species can also lead to a switch in ecosystem functions as original pond substrate transitions into sediment. This is an important finding for constructed ponds, demonstrating the potential for these features to be naturally engineered for OC storage and burial efficiency, through planting of certain vegetation species, to expedite the process of natural succession and promote conditions, which enhance OC burial.

These rates are some of the highest reported for semi natural aquatic environments and are substantially higher than published burial rates for forests, grasslands and a number of other terrestrial habitats. In comparison to rates recently published on emissions from very small ponds, which suggest they are a substantial source of CO_2 flux, it become apparent that in fact the balance is much closer than has previously been understood. Considering this alongside recent research identifying climate driven factors affecting C flux rates, in particular pond drying and rewetting, it is likely that this balance is heavily reliant upon, and responds both dynamically and rapidly to local climatic and environmental conditions.

1.3 Physicochemistry, Productivity and Hydrological Dynamics

The newly constructed ponds were monitored at roughly fortnightly intervals over a 3 year period for physicochemical variables including depth, NO_3^--N, PO_4^{3-} and chlorophyll-*a*. This data represents some of the highest resolution monitoring of physicochemistry of small constructed ponds, particularly temporary ponds with a recurrent dry-phase. All three of the ponds were hyper-eutrophic with concentrations of

chlorophyll-*a* > 40 µg L^{-1}, PO_4^{3-} > 100 µg L^{-1} and NO_3^--N >1500 µg L^{-1}. The ponds also demonstrated that increased nutrient concentrations in an already saturated system, result in the enhancement of productivity. Hyper-eutrophism was largely driven by avian inputs from the surrounding watershed amplified by the relatively small volume of the ponds in relation to larger water bodies.

Results also highlighted physicochemical variability between ponds driven by the proportion of nutrients entering the pond via stochastic deposition of faecal matter and the locality of the pond in relation to surface run-off from the surrounding site. Idiosyncratic behaviour was also observed in the physicochemical functioning of the ponds, largely driven by the individuality of pond hydro-period in relation to its immediate locality within the field. ST1 often retained water in warmer periods, whilst the ST2 and ST3 dried, resulting in enhanced productivity from decreased dilution and magnification of nutrient inputs. Pulses in productivity were also observed, particularly in ponds ST2 and ST3 upon rewetting after dry-phase. Dry-phases also had a substantial effect on pond physicochemistry, as it facilitated the initial establishment of *L.riparium* and *Agrostis*. *L.riparium* seems to have a pronounced effect on the pond substrate keeping conditions damp and promoting OC burial, especially across this early stage of succession, with ST2 and ST3 displaying characteristic sediment profiles, albeit storing low levels of OC.

Depth in individual ponds could be explained to a large degree by local climate variables. Climate had a pronounced effect on pond hydro-period with distinct differences observed between years of study in relation to the number and duration of dry-phases. Climate somewhat accounted for the distinct annual variations observed in the physicochemical functioning of the ponds between the different study years. Perhaps the most pronounced change was that observed across 2015 prior to the site flooding, where concentrations of nutrients and chlorophyll-*a* were much lower than previous years and displayed a markedly lower degree of variability. Such a pronounced change was attributed to the succession of the ponds as terrestrial vegetation communities began to encroach and establish within the ponds.

1.4 Microbial Ecology

Data from chapter VII provides novel information on bacterial community structure within small constructed ponds. The analysis has revealed significant differences in communities from groups of ponds, differentiated by past vegetation succession and by date of construction. Variations in bacterial community structure were most significant between upper and lower core layers reflecting the transition of newly accumulated

sediment into rooted layers of clay backfill. Complex and variable relationships were also observed between microbial community composition, vegetation coverage and sediment physicochemistry. Ponds dominated by *Juncus* species with wetter, looser and OC rich sediments displayed a higher abundance of facultative anaerobic microbial communities, whilst ponds with dense sediments, storing more OC supported communities dominated by more aerobic and drough resistant bacteria.

The *Proteobacteria* phylum dominated relative abundance in all samples. These phylum are well documented as being heavily involved in the biogeochemical cycling of important elements, including carbon. Other dominant phyla observed and documented as playing an important role in the transformation of both labile and recalcitrant OM were *Actinobacteria* and *Bacteroidetes*. Classes of methanogenic archaea were also observed within the ponds displaying distinct patterns between ponds and down the core profile. Considering these assemblages observed it is clear that the sediment within the ponds has the potential to be extremely active in terms of carbon and nutrient cycling.

Results have provided rare insights into the differences in microbial communities between newly constructed and mature ponds, a novel study that highlights the impact of pond succession on microbial community development. One of the most significant differences was that observed in the overall abundance of *Proteobacteria*, which was considerably less in the newer ponds. This suggests that recently accumulated sediment within the new ponds, which is known to store substantially less carbon than the mature ponds, may not be as metabolically active in terms of carbon cycling than the mature ponds.

Considering the potential for the ponds to switch between a net source and net sink of carbon to the atmosphere, under changing environmental conditions, highlighted in a study from Gilbert et al., (2016). Further research investigating the response of microbial communities and activity across changing environmental gradients, particularly climate driven hydro-period, developing linkages to carbon cycling and would provide more insightful information to identify microbial responses in small ponds to climate change.

It is also worth noting that ponds represent the nexus point between the terrestrial biosphere, the atmosphere and the hydrological cycle. Their small size promotes highly dynamic behaviour and enhanced ecosystem functioning and biogeochemical processing but leaves them highly susceptible to local climate. Therefore, they will be one of the first sytems to respond to changing climate.

2.0 Do Artificially Constructed Ponds Offer Any Potential as a Future Sink and Store of Carbon?

This study has identified that OC burial rates in artificially constructed ponds are substantially higher than those observed in the surrounding terrestrial area and other larger water bodies. The ponds were constructed in a small field located in a nature reserve and so have not been subject to agricultural run-off or management activities associated with substantial burial rates observed in other studies on small water bodies (Boyd, 2010; Downing 2010; Downing et al., 2008). Despite this, they still exhibit OC burial rates comparable to agricultural impoundments and aquaculture ponds that are subject to considerable anthropogenic influence.

So do artificially constructed ponds offer any potential as a future sink and store of carbon? The following section will assess the carbon mitigation potential from the micro-scale perspective of the Hauxley experimental site, extrapolating values to assess the potential at a national level for UK agriculture.

The Hauxley site occupies an area of roughly 12,498m^2 and currently holds 30 small mature ponds amongst the two larger permanent ponds and a number of newly constructed ponds that were constructed as part of this study. Table VIII.1 below indicates the amount of OC buried annually and that which would be stored over a 20 year period under a number of hypothetical scenarios, in which a set percentage of the land area had been utilised for the construction of more ponds.

Table VIII.1 Hypothetical estimates for land turnover at Hauxley for constructed ponds

	Land Area (m^2)	Carbon burial (t)	$tCO_2e\ yr^{-1}$	Storage over 20 yrs (t)	tCO_2e
Hauxley	12,498				
% of Land turnover					
10%	1249.8	0.18	0.65	3.56	13.07
20%	2499.6	0.36	1.31	7.12	26.13
50%	6249	0.89	3.27	17.80	65.33

The results demonstrate that under the proposed land turnover scenarios the ponds could potentially bury between 0.65 – 3.27 $tCO_2e\ yr^{-1}$, which over the course of 20 years the amount of carbon stored would equate to 13.07 - 65.33 $tCO_2e\ yr^{-1}$. Although these figures are pale in comparison to estimated GHG emissions from UK agriculture, which are projected to be in the region of around 56.1 $MtCO_2e\ yr^{-1}$ (DECC, 2015).

Article 3.4 of the Kyoto protocol of the UNFCCC has generated broad interest in the possibilities of using agricultural land for CO_2 mitigation. UK agriculture also has a binding commitment to reduce GHG emissions by 80% by 2050 (relative to 1990 levels) (Lamb et al., 2016). An 80% reduction in emissions would amount to around 20.8 $MtCO_2e$ and would require a range of measures to be implemented such as land sparing and changing behaviour such as reducing meat consumption (Lamb et al., 2016). Measures such as the industry action plan have also been introduced, which aims to reduce emissions by around 3 $MtCO_2e$ per year by 2020, representing a 34% reduction in emissions from 1990 levels (Agricultural GHG Action Plan: Framework for Action, 2010; Economics Group Defra, 2011)

Lamb et al., (2016) explored the idea of using land sparing to offset agricultural GHG emissions and proposed increasing UK forest cover from 12% to 30% and the restoration of up to 0.7Mha of peatlands. Here, we will propose a land sparing and turnover scenario based on 1% of the UK agricultural land area (17.2Mha, Defra, 2012).

Table VIII.2 Hypothetical estimates for agricultural land turned over for the construction of ponds

UK Agriculture	Land Area (km^2)	Carbon burial (t)	$MtCO_2e$ yr^{-1}	Storage over 20 yrs (t)	$MtCO_2e$
Total Land Area	172000				
1% Agricultural Area	1720				
10%	172	24499	0.09	489993.60	1.80
20%	344	48999	0.18	979987.20	3.60
50%	860	122498	0.45	2449968.00	8.99

The scenarios outlined above indicate that sparing just 1% of agricultural land and constructing artificial ponds to cover 10% to 50% of that land area could result in the substantial offset of agricultural GHG emissions. Annual burial rates of between 0.09 and 0.45 $MtCO_2e$ yr^{-1} could be expected by constructing ponds on 10 to 50% of that land area, resulting in the contribution of 3% - 15% of the 3 $MtCO_2e$ yr^{-1} reduction targets. Over a twenty year period this would amount to an overall storage of between 1.80 and 8.99 $MtCO_2e$ yr^{-1} resulting in the offset of substantial amounts of carbon.

Results outlined in this study suggest that OC burial and storage within the ponds is negligible over an initial three year period until the establishment of terrestrial based plant species such as *L.riparium*, which keeps sediment damp during drying events, preserving accumulated carbon and facilitating the development and accumulation of sediment. Ponds that exhibited early establishment of these species stored significantly

more OC. Informal discussions with pond managers throughout Europe that practice dredging to promote submerged macrophyte species, suggest that even after dredging, vegetation growth is usually rapid the following season. Within a couple of years ponds soon fill up with sediment, likely due to the existing seed bank and reduced competition for nutrients, which allows the rapid recolonisation of certain species. It is therefore possible that ponds created for carbon mitigation purposes could be planted or essentially “spiked”, with specific seed banks to promote their earlier establishment and faster succession, increasing their effectiveness in the burial of OC. Once the ponds have filled with sediment, there is also the option to remove this and use the nutrient rich sediment to improve the quality of arable soils and replace that lost from erosion.

The UK already has an obvious mechanism in place to promote the construction of ponds and implement such measures, through the EU’s Common Agricultural Policy. The policy has already implemented measures such as “greening” which require farmers to implement certain practices such as hedgerow creation, leaving land fallow and the creation of buffer strips next to water courses, if there land meets certain criteria. However, currently there are no mechanisms in place to encourage the construction of small ponds and wetlands. Surely given the results outlined above the construction of ponds should be considered as a greening option for farmers, particularly given their ease of construction, versatility and ability to provide a whole raft of other ecosystem services.

2.1 Call for Inclusion in Current Policy Mechanisms

Another emerging problem within the UK, in both agricultural and urban areas, is flooding. Planned Government spending on flooding is the highest it has been at years at a staggering £2.3bn up to 2021. This has recently been given an extra £700m boost to increase community protection from flooding. In the 2012 floods around 42,000ha of agricultural land was affected, causing an estimated £50million in damage (DEFRA, 2016). Recent research has emerged that constructing a network of strategically placed ponds can intercept all of the flow from a 25ha field drainage network, with no outflow (Biggs, 2007 report to DEFRA). Modelling and experimental studies have demonstrated that large reductions can be made in water loss from strategically placed basins, on the principle of installing 10,000m^3 of storage capacity (about ten medium sized ponds) to capture all of the rainfall in a heavy rainfall event within that km^2 (Biggs, 2007; Quinn, 2007). Coincidently this equates to roughly 1% of the land area within that km^2 similar to that outlined in the above scenario. Strategically placed ponds could therefore be used as both carbon mitigation tools and as natural flood water attenuation features.

The use of ponds as tools for water quality improvement is also well documented with a recent report from DEFRA highlighting the role of constructed, on farm wetlands in the mitigation of agricultural pollutants and capturing suspended solids (Newman et al., 2015). The report acknowledged that wetlands also provide other ecosystem services such as GHG mitigation, flood control and biodiversity improvement.

Another key issue for UK agriculture is the problem of soil erosion reducing the quality of arable farmland, which ultimately threatens the future production of crops. A recent parliamentary review on soil health states that some of the most productive agricultural land in the country is at risk of becoming unprofitable within a generation due to soil erosion and loss of organic carbon (House of Commons, 2016). The government has said that it would ensure the sustainable management of all soils by 2030 but the report outlined in their inquiry that current actions will not meet this ambition and highlighted doubts that the goal will be realised by 2030.

Considering the future goals and commitments of the UK Government to achieve a number of agricultural industry and environmental policy objectives, alongside the ability of ponds to provide a viable mechanism to achieve these, it is hard to understand why the construction of these features has received little attention as viable policy mechanisms, particularly under the CAP. In a similar fashion to the requirement of new urban developments to implement SuDs systems, why not implement similar systems across rural landscapes. The EA recently published a report on Rural Sustainable Drainage Systems (RSuDS) acknowledging that this is not a new concept and that the adoption of such systems has been relatively poor despite the growing opportunities for its implementation (Avery, 2012).

Ponds are relatively cheap and easy to create. Given the huge spending in achieving a number of policy objective outlined previously, particularly in regards to flooding, it is clear that ponds could be an ecologically effective and economically efficient tool in the construction of sustainable and resilient landscapes. Not many ecological policy tools have the ability to provide such a diverse range of services that and offer the natural sustainable advantages that ponds deliver. They harbour disproportionate species richness and rates of biogeochemical cycling that result in the significant improvement of wastewaters and store substantial amounts of OC.

It is the author's firm belief that the strategic construction of networks of small ponds across rural and urban areas alike should be encouraged, as tools in the construction of sustainable future landscapes that are resilient to the increasing frequency of extreme weather events and ultimately, future climate change.

3.0 Conclusion

Small ponds are currently omitted from conceptual global carbon cycles and inventories. If the carbon cycle is to be comprehensively and accurately quantified, biogeochemical cycling of sub compartments such as ponds, need to be integrated with top down approaches and global circulation models. Further studies like the one presented here, quantifying micro-scale field processes and elucidating factors influencing the magnitude of processing rates, are required to facilitate the production of representative and accurate quantification of carbon cycling within these small systems.

Their important designation as a sink/source is a long way from being established and is a hugely important element in determining their role and function within the carbon cycle and implementation as carbon mitigation features. However, burial rates produced within this study can act as a useful comparison to published flux data, particularly a recent study by Holgerson & Raymond (2016) which suggests very small ponds are a significant source of CO_2 to the atmosphere. Factoring in accumulation rates of OC it becomes clear that in fact the balance between functionality as a sink/source is much closer than has previously been identified.

Other studies have revealed dynamic and almost instantaneous response in carbon emission driven by changing hydrological conditions. It is therefore likely that the sink/source balance may well be governed by environmental and hydrological conditions, driven by local climate. If climatic conditions govern the functional role of small water bodies within the global carbon cycle, it may well be that small water bodies are a dynamic sub-compartment in a climate controlled equilibrium, switching between sink and source. If this is the case, developing a critical understanding of feedback mechanisms, in response to future climate projections is necessary, if the role of this dynamic sub-compartment is to be fully realised.

Considering results from this study at a more landscape scale is interesting. The high rates of OC burial and accumulation over a relatively short life span (~20yrs) yields exciting implications for their construction and implementation as carbon mitigation tools. Burial rates published here are some of the highest reported for natural aquatic systems and are substantially higher than those reported for terrestrial ecosystems. Although OC storage in the sediment appears similar to the surrounding terrestrial area, what is interesting and most significant is the rate at which this carbon is accumulated, at least double the rate of the surrounding soil. It should also be highlighted that the burial rates are conservative, in that, the majority of sediment is

likely accumulated within the mid-late succession phase of the pond, as identified in the comparison of carbon storage between mature and new ponds.

The influence of vegetation establishment on carbon storage has also been identified indicating that the earlier establishment of certain species, and ultimately faster transition through succession stages, promotes increased OC storage. The potential for these constructed systems to be further enhanced and managed for carbon capture is evident. Alongside changing Government policy and legislation on agricultural subsidies and responsibilities for biodiversity, the creation and implementation of ponds as ecologically and economically efficient tools in the construction of sustainable landscapes is a realistic option. When this is considered alongside other ecosystem services provided such as floodwater attenuation, water quality improvement and soil retention, their ability to achieve a whole suite of environmental policy objectives can be realised and should therefore be considered or better still included, in current and future policy mechanisms.

Ponds have captured the hearts and minds of many throughout history dating as far back as the 4th Century BC. Aristotle noted the dynamic nature of these systems, describing the “seemingly spontaneous generation of life from rain, mud or sand”. Much more recently, their ecological importance has been revealed, offering refuge to a number of rare and red data book listed species. Across landscapes, these aquatic islands surrounded by seas of terrestrial habitat, lead to the formation and development of unique and highly individual communities. Their intrinsic relationship to local climate will undoubtedly leave them one of the first and most dynamic ecosystems to respond to climate change, a pattern already observed in Mediterranean Temporary Ponds. They form the nexus point of exchanges between aquatic, riparian, terrestrial and atmospheric processes, demonstrating disproportionately intense productivity and biogeochemical cycling that has until recently been overlooked in favour of larger ecosystems. It is hoped that this research may act as a springboard for future research to establish the true extent and importance of these small yet highly functional ecosystems.

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Appendices

Table IX.1: List of ecosystem services by category for wetlands. Taken from Maltby and Omerod 2011 National Ecosystem Assessment Technical report Ch9 freshwaters.

Provisioning	**Conditions or characteristics of habitats required**
Fish	Commercially significant fisheries (crayfish, salmon, trout) based on rivers, lakes and ponds in suitable conditions
Reeds, osiers and watercress	Reeds grow in saturated soils and slow flowing or still water up to 0.3m deep. Osiers produce withies for basket making; requiring saturated soil conditions. Cress-beds need swiftly flowing high pH clean water
Water	Open water habitats provide a water source for public supply, irrigated crops, power station cooling, industrial processing and fish farming, but high evaporation rates may suppress total water availability
Peat	Peat provides the basis of some composts for horticulture. Peat needs to be >0.5m deep to be commercially exploitable due to recent planning guidance
Health products	Mineral spas, medicinal plants (E.g. bogbean), medicinal leeches
Regulating	
Carbon regulation	Carbon accumulates where production of plant litter exceeds decomposition and generally under waterlogged, predominantly anaerobic conditions. Deposition of organic sediments within lakes, ponds and reservoirs is an important component of the carbon budget
Flood regulation	Flood reduction relies on available water storage. Permanently saturated habitats with no storage may generate or augment floods
Flow regulation	River flow, groundwater recharge influenced by landscape location, water storage characteristics and connection with other water bodies
Water quality regulation	Freshwater systems can dilute, store and detoxify waste products and pollutants, however there are threshold levels and some systems may accumulate substances to toxic levels
Local climate regulation	Temperature and humidity may be different within the habitat and without; degree depends on size. Important moist microclimates can develop
Fire regulation	Open water bodies can act as natural fire breaks
Human health regulation	Natural freshwater systems can increase well being and quality of life if visually attractive and supportive of physical recreation. Mismanaged freshwaters can be sources of water borne diseases and disease vectors (e.g. mosquitoes), but also sources of bio control agents
Cultural	
Science and education	Lake, floodplain and mire sediment sequences contain palaeo-environmental archives and human (pre)history, artefacts that may be lost if disrobed or desiccated. Freshwater ecosystems are important outdoor laboratories
Tourism and recreation	Extensive recreational fisheries (game species and coarse fisheries depend on good habitat). Tourism depends on landscape appeal and iconic species, such as rare birds, flowers or amphibians. Good water quality and visual appearance required for natural swimming and boating
Sense of place	Water is important in defining specific landscape character and features strongly in art and local culture. Literary and cultural identities embodied in distinctive landscapes such as Snowdonia, the Lake District, the Somerset Levels, Gwent Levels or the Norfolk Broads
History	Freshwaters and especially wetlands have played a key role in human history and settlement since prehistoric times. Water is a recurrent feature at the heart of many historically important places, battlefields, territorial boundaries and many local folklore connections
Supporting Services	
Biodiversity	All freshwater habitats with open water: species depend on conditions such as temperature, oxygen level, depth and velocity of water and area with suitable conditions. Some habitats may provide temporary habitat for fish (e.g. spawning), such as floodplains

Figure IX.1: a) Vegetation community succession amongst the Hauxley ponds years 1995-2001.

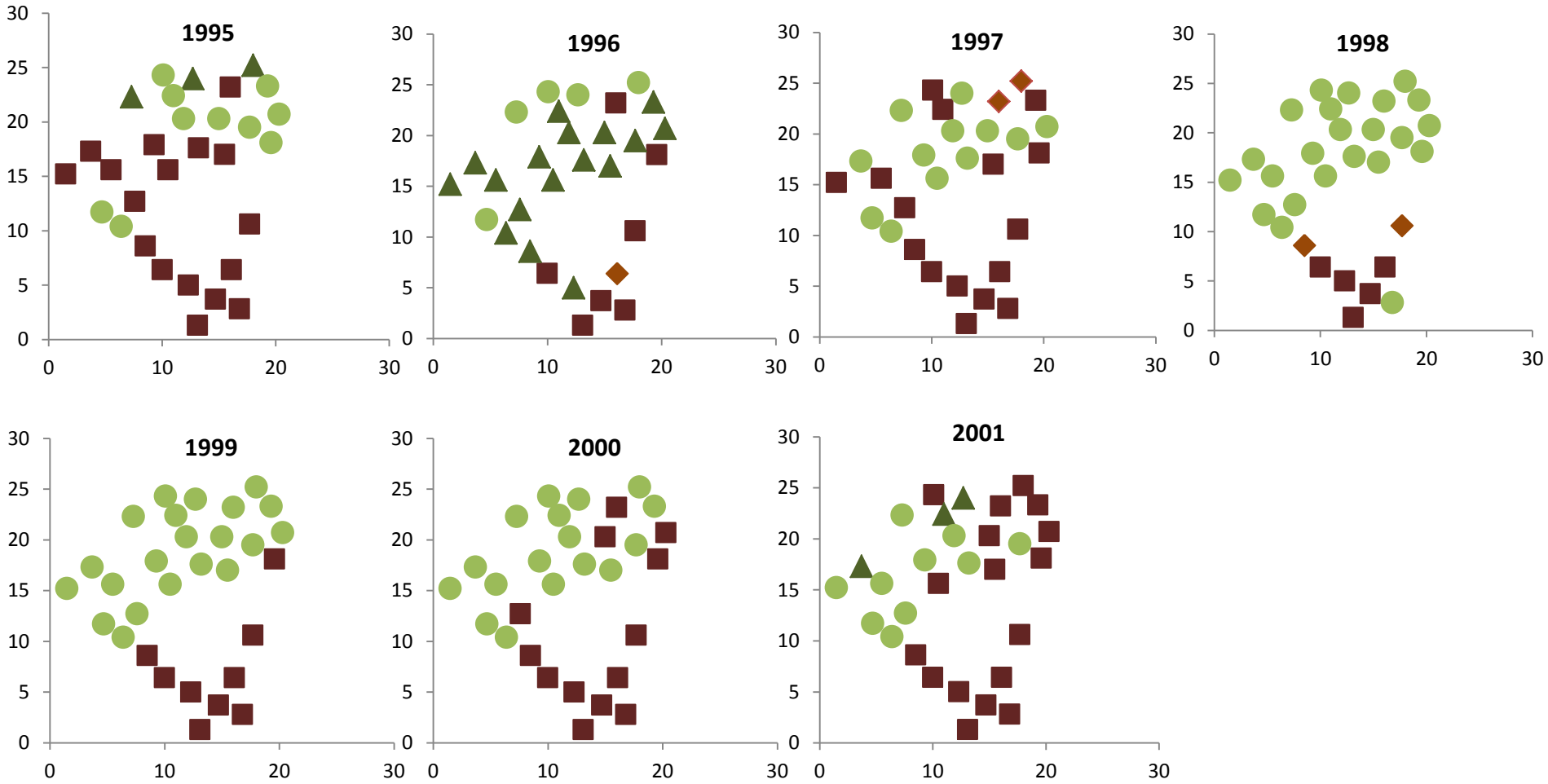


Figure IX.1: b) Vegetation community succession amongst the Hauxley ponds years 2002 – 2008.

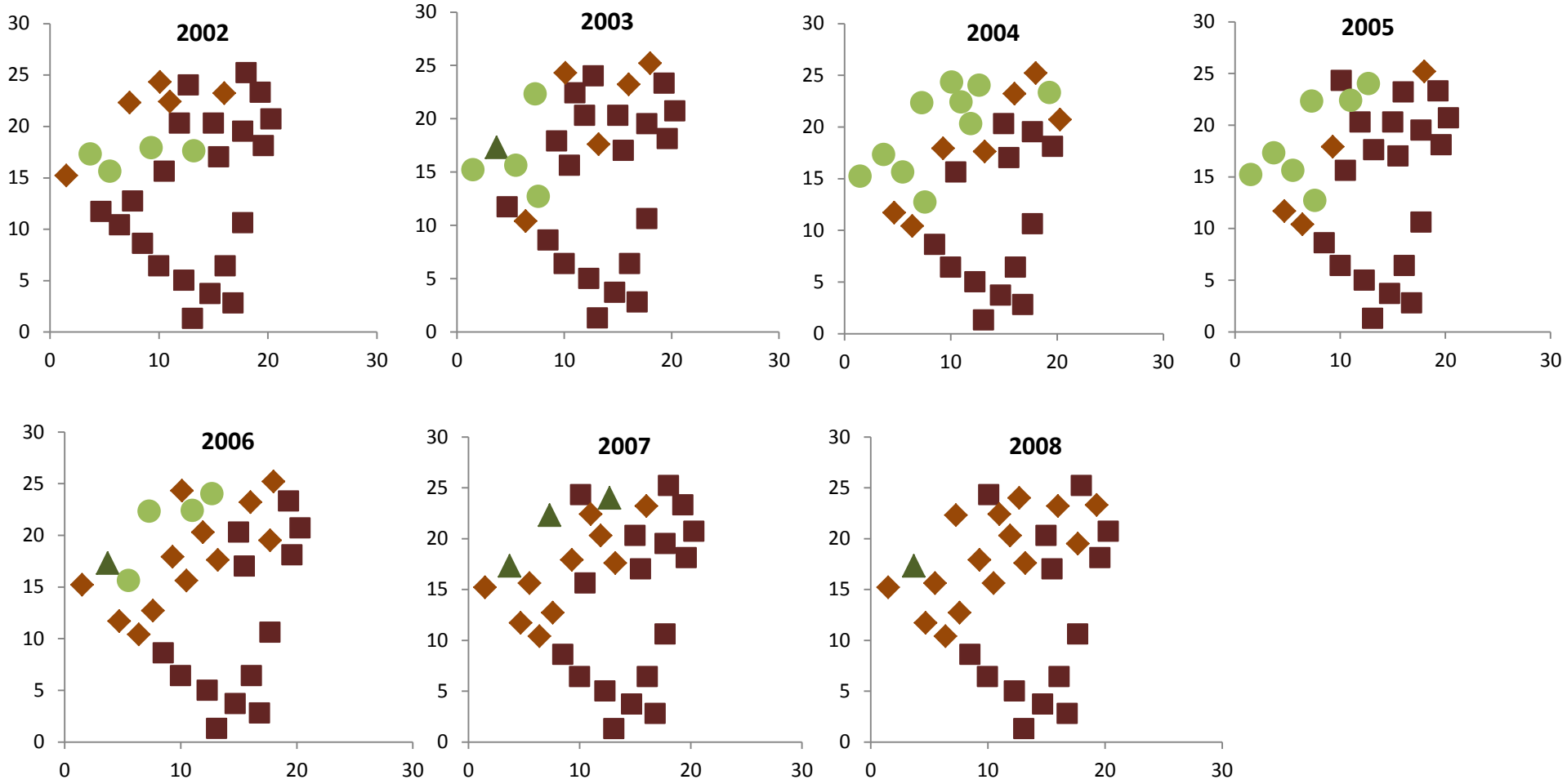
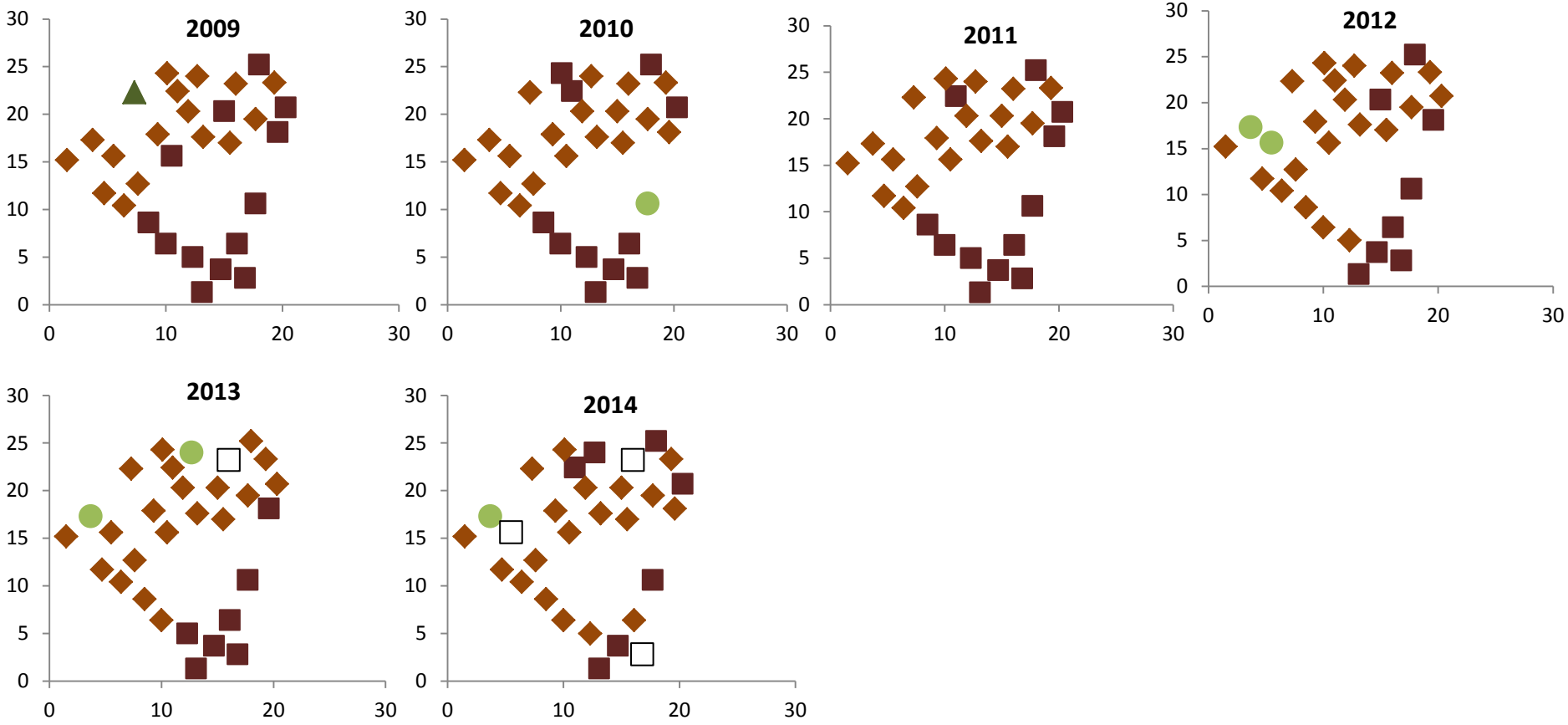


Figure IX.1: c) Vegetation community succession amongst the Hauxley ponds years 2009 – 2014.



Worked Example of Sediment Core Extrapolation

DCA analysis has identified the sediment-clay interface as 5cm.

Physicochemical variables identified for identified sediment layers are presented and calculated in the table below:

Sediment Depth Layer (cm)	OC%	DBD ($g\ cm^{-3}$)	C Density ($mg\ OC\ cm^{-3}$)	Volume of sediment layer in core ($\pi\ r^2$ x sediment depth) (r =core diameter / 2) (Core diameter = 4.7cm)	Carbon Stock in individual sediment layer (g OC)
1	20	0.25	50	17.35	0.87
2	12	0.5	60	17.35	1.04
3	10	0.75	75	17.35	1.30
4	8	1.0	80	17.35	1.38
5	6	1.25	75	17.35	1.30
Sum					5.89

Sum of carbon stored in whole sediment core = 5.89 g OC

Carbon stored per cm^2 of sediment = 5.89 / 17.35 (surface area of corer) = 0.3395

Carbon stored per m^2 ($g\ OC\ m^{-2}$) = 0.34 x 10000 = 3394.81 g OC m^{-2}

If this pond was constructed 20 years prior to this core being taken:

Carbon Burial = 3394.81 / 20 = 168.74 g OC $m^{-2}\ yr^{-1}$

Table IX.2: Simper results top 30 OTU's responsible for dissimilarity between community compositions of mature and new ponds. OTU's highlighted in bold represent a higher abundance within the mature ponds.

Simper	Mature Ponds	New Ponds			
Name	Ave Abund	Ave Abund	Ave Dissim	% Contribution	Cumulative %
Gammaproteobacteria;Aeromonadales;Aeromonadaceae; Aeromonas;	**1069.74**	**24.17**	**1.00**	**1.10**	**1.10**
Spirochaetes;Spirochaetales;Spirochaetaceae;unclassified;	0.00	948.50	0.93	1.02	2.12
Bacteroidetes;unclassified;	53.62	792.33	0.73	0.81	2.93
unclassified;	314.48	533.83	0.65	0.71	3.64
Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis;	22.97	971.33	0.58	0.64	4.28
unclassified;	162.54	513.00	0.48	0.53	4.81
Gammaproteobacteria;Alteromonadales;Shewanellaceae; Shewanella;	**521.70**	**2.17**	**0.48**	**0.52**	**5.33**
Acidobacteria;Acidobacteria_Gp4;Acidobacteria_Gp4_order_incertae_sedis;	10.84	792.33	0.43	0.47	5.80
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified;	8.57	605.83	0.40	0.44	6.24
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae; Pseudomonas;	**364.90**	**64.33**	**0.39**	**0.43**	**6.67**
Bacteroidetes;unclassified;	49.06	443.50	0.38	0.42	7.09
unclassified;	1.85	330.67	0.37	0.41	7.49
Deltaproteobacteria;Desulfobacterales;Desulfobulbaceae;unclassified;	0.46	481.33	0.36	0.40	7.89
Gammaproteobacteria;Pseudomonadales; Pseudomonadaceae;Pseudomonas;	**359.44**	**0.00**	**0.36**	**0.40**	**8.29**
Gammaproteobacteria;unclassified;unclassified;unclassified;	10.36	501.00	0.36	0.39	8.68
Betaproteobacteria;unclassified;unclassified;unclassified;	138.45	607.17	0.35	0.39	9.07
Acidobacteria";Acidobacteria_Gp16;Acidobacteria_Gp16_order_incertae_sedis;	107.49	613.00	0.35	0.39	9.46
Bacteroidetes;unclassified;	**334.98**	**0.00**	**0.34**	**0.37**	**9.84**
Bacteroidetes;"Bacteroidia";"Bacteroidales";"Marinilabiaceae";Alkaliflexus;	0.00	524.00	0.33	0.37	10.21
Verrucomicrobia;Subdivision3;Subdivision3_order_incertae_sedis;	**503.46**	**256.17**	**0.33**	**0.37**	**10.57**
Bacteroidetes;unclassified;	0.04	651.00	0.31	0.34	10.92
Betaproteobacteria;Hydrogenophilales;Hydrogenophilaceae; Thiobacillus;	103.67	251.17	0.30	0.33	11.25
Bacteroidetes;unclassified;	5.62	294.17	0.30	0.33	11.58
Bacteroidetes;Sphingobacteria;Sphingobacteriales;Cyclobacteriaceae;unclassified;	0.02	496.00	0.29	0.33	11.91
Actinobacteria;Actinobacteria;Actinomycetales;Intrasporangiaceae;Phycicoccus;	28.37	513.33	0.29	0.32	12.23
Bacteroidetes;unclassified	0	260.83	0.29	0.32	12.55
Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis	**345.99**	**108.83**	**0.29**	**0.32**	**12.87**
Gammaproteobacteria;Xanthomonadales;unclassified	2.75	349.33	0.28	0.31	13.18
Chloroflexi;unclassified	27.63	402	0.28	0.31	13.49
Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis	17.15	523.67	0.28	0.31	13.80

Table IX.3: Simper results top 30 OTU's responsible for dissimilarity between community compositions between upper and lower sediment core layers. OTU's highlighted in bold represent a higher abundance within the lower layers to the upper layers.

	Lower Layers Ave Abund	Upper Layers Ave Abund	Ave Dissim	% Contr-ibution	Cumu-lative %
Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Aeromonas	34.90	1876.92	2.03	2.27	2.27
Gammaproteobacteria;Alteromonadales;Shewanellaceae;Shewanella	137.33	821.50	0.98	1.10	3.37
unclassified	**717.67**	**0.00**	**0.80**	**0.90**	**4.26**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	**589.39**	**189.80**	**0.79**	**0.88**	**5.15**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	54.54	597.26	0.74	0.83	5.97
Bacteroidetes;unclassified	21.23	579.70	0.69	0.77	6.75
Proteobacteria;unclassified	46.82	468.32	0.54	0.60	7.35
Deltaproteobacteria;unclassified	**504.74**	**84.68**	**0.51**	**0.57**	**7.92**
Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis	**509.33**	**218.58**	**0.48**	**0.53**	**8.45**
unclassified	**287.69**	**227.10**	**0.43**	**0.48**	**8.94**
Bacteroidetes;unclassified	10.97	370.66	0.42	0.47	9.40
unclassified	**364.13**	**5.30**	**0.40**	**0.44**	**9.84**
Gammaproteobacteria;unclassified	**334.59**	**33.94**	**0.37**	**0.42**	**10.26**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	0.00	339.84	0.36	0.41	10.67
Verrucomicrobia;Subdivision3;Subdivision3_order_incertae_sedis	475.54	525.24	0.36	0.40	11.07
Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geobacter	**376.87**	**70.40**	**0.36**	**0.40**	**11.47**
Bacteroidetes;unclassified	**307.26**	**0.00**	**0.34**	**0.38**	**11.86**
unclassified	**318.08**	**12.08**	**0.33**	**0.37**	**12.23**
Betaproteobacteria;Rhodocyclales;Rhodocyclaceae;unclassified	118.10	323.14	0.32	0.35	12.58
unclassified	**293.21**	**20.48**	**0.31**	**0.35**	**12.93**
unclassified	**286.39**	**37.52**	**0.30**	**0.34**	**13.27**
unclassified	**259.82**	**5.72**	**0.30**	**0.33**	**13.60**
unclassified	**271.46**	**10.48**	**0.29**	**0.33**	**13.93**
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified	**240.80**	**4.72**	**0.28**	**0.31**	**14.24**
Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis	7.15	247.92	0.27	0.30	14.54
Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;unclassified	39.00	271.86	0.27	0.30	14.84
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	**215.49**	**0.80**	**0.26**	**0.29**	**15.13**
Gammaproteobacteria;unclassified	34.08	248.72	0.25	0.28	15.41
Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Yersinia	0.64	196.82	0.23	0.25	15.66
Betaproteobacteria;Hydrogenophilales;Hydrogenophilaceae;Thiobacillus;	85.87	232.90	0.22	0.25	15.91
Verrucomicrobia;Subdivision3;Subdivision3_order_incertae_sedis	97.54	192.96	0.21	0.23	16.14

Table IX.4: Simper results top 25 OTU's responsible for dissimilarity between community compositions between upper and lower sediment core layers. OTU's highlighted in bold represent a higher abundance within the Group 1 ponds.

	Group1 Ave Abund	Group2 Ave Abund	Ave Dissim	% Contr-ibution	Cumu-lative %
Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Aeromonas	**1692.0**	**1379.76**	**2.74**	**3.37**	**3.37**
Gammaproteobacteria;Alteromonadales;Shewanellaceae;Shewanella	202.07	1085.93	1.29	1.59	4.96
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	311.07	719.55	1.05	1.30	6.26
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	225.93	690.52	0.92	1.14	7.40
Bacteroidetes;unclassified	**422.20**	**343.07**	**0.61**	**0.75**	**8.15**
unclassified	332.07	403.35	0.58	0.71	8.86
unclassified	266.17	358.10	0.49	0.61	9.46
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas;	160.50	264.48	0.46	0.56	10.03
Bacteroidetes;unclassified	209.93	308.79	0.43	0.54	10.56
Acidobacteria;Acidobacteria_Gp;Acidobacteria_Gp6_order_incertae_sedis	357.27	403.10	0.43	0.53	11.10
Deltaproteobacteria;unclassified	**277.40**	**242.83**	**0.42**	**0.52**	**11.62**
Proteobacteria;unclassified	80.67	357.45	0.40	0.50	12.12
Bacteroidetes;unclassified	**326.87**	**70.14**	**0.37**	**0.45**	**12.57**
Verrucomicrobia;Subdivision3;Subdivision3_order_incertae_sedis	**512.70**	**505.48**	**0.36**	**0.44**	**13.01**
unclassified	**316.83**	**80.21**	**0.34**	**0.42**	**13.42**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	0.00	288.17	0.33	0.41	13.84
Gammaproteobacteria;unclassified	171.13	231.17	0.33	0.41	14.25
unclassified	**252.27**	**139.10**	**0.33**	**0.41**	**14.65**
Betaproteobacteria;Rhodocyclales;Rhodocyclaceae;unclassified;	236.03	355.90	0.31	0.38	15.04
Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Yersinia	0.87	253.07	0.31	0.38	15.41
Chloroflexi;Anaerolineae;Anaerolineales;Anaerolineaceae;unclassified	**167.60**	**130.38**	**0.28**	**0.35**	**15.76**
unclassified	**239.63**	**14.21**	**0.26**	**0.31**	**16.08**
Betaproteobacteria;Hydrogenophilales;Hydrogenophilaceae;Thiobacillus;	**232.37**	**61.55**	**0.25**	**0.31**	**16.39**
Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geobacter;	**223.93**	**166.90**	**0.25**	**0.30**	**16.69**
Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;unclassified;	**201.17**	**145.14**	**0.24**	**0.30**	**16.98**
unclassified	**150.83**	**135.62**	**0.23**	**0.29**	**17.27**

Table IX.5 Simper results top 25 OTU's responsible for dissimilarity between community compositions between upper and lower sediment core layers. OTU's highlighted in bold represent a higher abundance within the Group 1 ponds.

	Group1 Ave Abund	Group3 Ave Abund	Ave Dissim	% Contr-ibution	Cumu-lative %
Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Aeromonas	**1692**	**147.8**	**2.00**	**2.35**	**2.35**
Bacteroidetes;unclassified	**422.2**	**239.933**	**0.61**	**0.72**	**3.07**
Gammaproteobacteria;Alteromonadales;Shewanellaceae;Shewanella	202.067	295.9	0.54	0.63	3.71
Proteobacteria;unclassified	80.6667	415.2	0.51	0.60	4.30
unclassified	**332.067**	**211**	**0.48**	**0.57**	**4.87**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	**311.067**	**59.7**	**0.48**	**0.56**	**5.44**
Deltaproteobacteria;unclassified	277.4	285.167	0.44	0.52	5.96
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae Pseudomonas	**225.933**	**189.1**	**0.44**	**0.51**	**6.47**
Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis	**357.267**	**279.5**	**0.40**	**0.47**	**6.95**
Bacteroidetes;unclassified	**326.867**	**4.76667**	**0.37**	**0.43**	**7.38**
unclassified	**266.167**	**140.167**	**0.36**	**0.43**	**7.81**
Verrucomicrobia;Subdivision3;Subdivision3_order_incertae_sedis	**512.7**	**492.267**	**0.35**	**0.41**	**8.22**
unclassified	**316.833**	**39.2667**	**0.34**	**0.40**	**8.62**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	**160.5**	**150.233**	**0.33**	**0.39**	**9.02**
unclassified	**198.3**	**176.833**	**0.33**	**0.39**	**9.40**
unclassified	**239.633**	**93.9333**	**0.32**	**0.38**	**9.79**
Bacteroidetes";unclassified	**209.933**	**123.6**	**0.32**	**0.38**	**10.16**
unclassified	**252.267**	**95.4667**	**0.31**	**0.37**	**10.53**
Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geobacter	**223.933**	**222**	**0.30**	**0.36**	**10.89**
Betaproteobacteria;Hydrogenophilales;Hydrogenophilaceae;Thiobacillus	**232.367**	**15.7**	**0.27**	**0.32**	**11.20**
Deltaproteobacteria;Desulfobacterales;Desulfobacteraceae;unclassified;	**201.167**	**162.333**	**0.26**	**0.31**	**11.52**
Betaproteobacteria;Rhodocyclales;Rhodocyclaceae;unclassified	**236.033**	**112.033**	**0.24**	**0.29**	**11.81**
Bacteroidetes;"Sphingobacteria;"Sphingobacteriales;Chitinophagaceae;unclassified	0.033333	212.267	0.24	0.28	12.09
Gammaproteobacteria;unclassified	**171.133**	**96.9333**	**0.23**	**0.27**	**12.36**
unclassified	**180.8**	**31.7333**	**0.23**	**0.27**	**12.63**
Betaproteobacteria;Hydrogenophilales;Hydrogenophilaceae;Thiobacillus	**209.633**	**191.367**	**0.23**	**0.27**	**12.90**

Table IX.6: Simper results top 25 OTU's responsible for dissimilarity between community compositions between upper and lower sediment core layers. OTU's highlighted in bold represent a higher abundance within the Group 2 ponds.

	Group2 Ave Abund	Group3 Ave Abund	Ave Dissim	% Cont-ribution	Cumu-lative %
Gammaproteobacteria;Aeromonadales;Aeromonadaceae;Aeromonas	**1379.76**	**147.8**	**1.57**	**1.90**	**1.90**
Gammaproteobacteria;Alteromonadales;Shewanellaceae;Shewanella	**1085.93**	**295.9**	**1.37**	**1.65**	**3.55**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	**690.517**	**189.1**	**0.89**	**1.07**	**4.62**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	**719.552**	**59.7**	**0.87**	**1.05**	**5.67**
Proteobacteria;unclassified	357.448	415.2	0.60	0.72	6.39
unclassified	**403.345**	**211**	**0.58**	**0.70**	**7.09**
Bacteroidetes;unclassified	**343.069**	**239.933**	**0.48**	**0.58**	**7.66**
Bacteria;unclassified	**358.103**	**140.167**	**0.46**	**0.56**	**8.22**
Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis	**403.103**	**279.5**	**0.44**	**0.53**	**8.75**
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	**264.483**	**150.233**	**0.42**	**0.51**	**9.26**
Verrucomicrobia;Subdivision3;Subdivision3_order_incertae_sedis	**505.483**	**492.267**	**0.39**	**0.47**	**9.73**
Gammaproteobacteria;Enterobacteriales;Enterobacteriaceae;Yersinia	**253.069**	**83.3667**	**0.38**	**0.46**	**10.19**
Bacteroidetes;unclassified	**308.793**	**123.6**	**0.37**	**0.44**	**10.63**
Deltaproteobacteria;unclassified	242.828	285.167	0.36	0.43	11.06
Gammaproteobacteria;Pseudomonadales;Pseudomonadaceae;Pseudomonas	**288.172**	**2.9**	**0.35**	**0.42**	**11.49**
Betaproteobacteria;Rhodocyclales;Rhodocyclaceae;unclassified	**355.897**	**112.033**	**0.35**	**0.42**	**11.91**
Gammaproteobacteria;unclassified	**231.172**	**96.9333**	**0.30**	**0.36**	**12.27**
Deltaproteobacteria;Desulfuromonadales;Geobacteraceae;Geobacter	166.897	222	0.28	0.34	12.60
Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;unclassified	1.7931	212.267	0.24	0.29	12.89
AcidobacteriaAcidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis	**159.828**	**128.1**	**0.23**	**0.28**	**13.17**
unclassified	**163.379**	**112.733**	**0.22**	**0.27**	**13.44**
Bacteroidetes;Sphingobacteria;Sphingobacteriales;Chitinophagaceae;Terrimonas	**187.276**	**137.133**	**0.22**	**0.27**	**13.71**
unclassified	61.7586	176.833	0.22	0.27	13.97
Betaproteobacteria;unclassified	73.2759	145.8	0.22	0.26	14.24
Acidobacteria;Acidobacteria_Gp6;Acidobacteria_Gp6_order_incertae_sedis	130.828	189.033	0.21	0.26	14.50
Bacteria;unclassified	37.8276	170.933	0.21	0.25	14.75